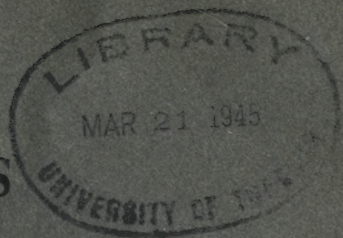


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STUDIES

FROM THE

ARTHUR S. A. SPRAGUE MEMORIAL  
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VOLUME VIII

CHICAGO  
1920





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1920





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TUBERCLE BACILLUS AND ON  
EXPERIMENTAL TUBERCULOSIS

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY  
OF TUBERCULOSIS. XIX

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# THE INFLUENCE OF CREOSOTE, GUAIACOL AND RELATED SUBSTANCES ON THE TUBERCLE BACILLUS AND ON EXPERIMENTAL TUBERCULOSIS

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY  
OF TUBERCULOSIS. XIX.

LYDIA M. DEWITT, BINZI SUYENAGA  
AND  
H. GIDEON WELLS

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Of the countless drugs used in tuberculosis, creosote and its derivatives undoubtedly hold first place in extent of use and general reputation, but absolutely without scientifically established value. Perhaps as good a summarization of their status as any is given by Lawrason Brown,<sup>1</sup> who says that creosote and its derivatives "are the most used of false specifics. They have never been proved to exert any action on the tuberculous process but in some patients have almost a specific action upon the accompanying secondary infection of the lungs, such as simple bronchitis. They also exert a very stimulating effect upon the bronchial mucous membrane during their excretion through it." Other authors vary somewhat in their judgment, but positive statements are avoided, of necessity. Kobert says, "Very little that is conclusive can be said concerning the usefulness of these preparations." Bandelier Ropke's "Die Klinik der Tuberkulose" contains the conclusion that "Creosote and guaiacol preparations are not internal disinfectants, but for certain cases they stimulate the appetite and improve digestion. Their routine use is therefore by no means justifiable."

A careful examination of the extensive literature on the many compounds of this class indicates that opinions as to their value or action, whether favorable or unfavorable, rest upon very slender evidence. General clinical experience is the only extensive source of information, and this is, of course, uncontrolled and conflicting, so it

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<sup>1</sup> Klebs: Tuberculosis, New York, D. Appleton & Co., 1909.

does not serve as a basis for conclusions of any value whatever. The most that can be said is that the use of creosote therapy in tuberculosis has persisted so long and so widely that it seems probable that some beneficial results must have been observed. Medical history gives us the right to suspect that any methods or substances of therapeutics that continue to be generally used for many years have some definite favorable effect, although the real establishment of their principles and limitations may not be understood. Sometimes after years of clinical use, and sometimes even after long subsequent years of disuse, the explanation of their activity has been established.

There has been no lack of endeavor by chemical manufacturers to provide all possible derivatives of creosote and its components, as can be seen in any list of chemical products, but especially in Fraenkel's "Arzneimittelsynthese." These endeavors have, with few exceptions, not been based on considerations of bactericidal efficiency, and indeed this essential aspect has been almost completely neglected. As Fraenkel says in summarizing this section of his book, two objects underlie the preparation of these multitudinous derivatives of creosote and guaiacol—(a) reduction of the toxicity and irritating properties, together with improvement in taste; (b) water solubility. Most of the products accomplishing the first objects, largely by means of the salol principle, are not water-soluble. The water-soluble compounds are mostly bad tasting, and, in case of the sulfonates, are much reduced in activity. "In therapeutic application, in the first rank of products indisputably come those formed by esterification of the hydroxyl group." He also comments that of the active components of creosote only the guaiacol has had general use in pure form, while the less toxic cresols, which have analogous effects, have had no consideration.

There are numerous compilations of literature on creosote therapy in tuberculosis (under which term are included the numerous derivatives of creosote and related substances), and we shall not give another compilation. One of the best reviews is that by von Weismayr in Ott's "Chemische Pathologie der Tuberculose." We give, however, in brief form such scanty evidence as we have found concerning the bactericidal action of this class of compounds, both *in vitro* and *in vivo*:

Bouchard is quoted by Weismayr as having found that 0.8 per 1,000 strength of creosote in glycerol bouillon retards the growth of tubercle bacilli, and 0.5 per 1,000 is effective in blood serum. In daily doses of 0.25 gm. per kilo it caused immunity to tuberculosis in rabbits, so that animals killed after three months showed no lesions. We regret that we have not been able to find the origin of this statement, for it is perhaps the most definite one in the literature.

On the other hand, Cornet<sup>2</sup> reported that he inoculated 7 guinea-pigs with tuberculosis after they had been given 0.02 gm. creosote daily per catheter into the stomach for about a month, and continued after infection, but they all died of tuberculosis as did the control animals. This dose he estimated as corresponding to 2.0 gm. creosote per day in a man. He also quotes Schüller as obtaining positive results and Sormani and Pallacani as obtaining negative results by causing infected guinea-pigs to inhale creosote.

Guttman<sup>3</sup> studied the inhibition of growth of different species of bacteria on gelatin containing creosote, which he found to be more effective than phenol. Of 18 species of bacteria, 12 failed to grow when the gelatin contained one part in 2,000 (including 4 not growing at 1:4,000), and 5 of the remaining 6 failed to grow when the concentration was 1:1,000. With 17 species on gelatin containing phenol, 12 grew when the concentration was 1:2,000. Tubercle bacilli were partly inhibited by creosote 1:4,000 and even 1:16,000, but it required 1:2,000 to prevent growth entirely. He then speculated as to the possibility of obtaining a concentration of 1:2,000 of creosote in the human body, and found that this could not be done, wherefore he was doubtful as to the efficiency of creosote in tuberculosis—a speculation that has been quoted extensively in literature on tuberculosis.

Fraenkel<sup>4</sup> corroborated in 1889 the statement that cresols are stronger antiseptics than phenol. He quotes von Behring's estimate that one-sixth the amount of an antiseptic found to be inhibiting for bacteria represents approximately the lethal dose for an animal. In accordance therewith, Fraenkel found that 1:300 cresol sulfate is inhibiting to bacteria (species not stated) which would correspond on this basis to two-sixths gm. in a 600 gm. pig. an amount found fatal although a slightly smaller dose was not fatal.

Kuprianow<sup>5</sup> quotes Petrescu as finding that tubercle bacilli did not grow in 10 cc bouillon containing 4.1 gm. (sic) guaiacol, and that tubercle bacilli exposed to guaiacol did not produce tuberculosis. Marfori also found that guaiacol rendered tubercle bacilli unable to infect rabbits. No other references to direct observations on the bactericidal power of guaiacol could be found in the literature to 1894 by Kuprianow. He therefore made some tests by Loeffler's method, in which the solution to be tested is poured over the surface of an inoculated agar slant, and poured off again after a specified time, the tube then being incubated. In other experiments 24 to 48 hour slant cultures were exposed to the solutions and material removed with a platinum loop was inoculated on fresh mediums. The inhibiting effect in bouillon cultures was also determined. Phenol and creosote were found by these methods to be about equally destructive to staphylococci and *B. pyocyaneus*, but guaiacol was less active than either. To all 3 the staphylococcus was much more resistant than the *pyocyaneus* bacilli. Tubercle bacilli were exposed in masses of culture to 4% alcoholic solution of guaiacol and creosote for 15 seconds to 2 hours, and after pouring off the solution no growth followed. Obviously these last experiments are too crude to be of any significance.

Koch is quoted as having found that creosote inhibits the growth of tubercle bacilli in cultures, but the reference cited in the literature is incorrect, and the original article has not been located.

<sup>2</sup> Ztschr. f. Hyg. u. Infektionskr., 1888, 5, p. 124

<sup>3</sup> Ztschr. klin. Med. 1888, 13, p. 488

<sup>4</sup> Ztschr. f. Hyg. u. Infektionskr. 1889, 6, p. 111

<sup>5</sup> Centrabl. f. Bakteriöl., 1894, 17, p. 933



Winkler<sup>6</sup> exposed agar plate cultures of tubercle bacilli to vapor from a mixture of guaiacol and iodoform for eight days and found that the material became noninfectious. Injection of this mixture into animals did not save them from tuberculosis, and serum of rabbits injected with the antiseptic had no effect on infection with tubercle bacilli.

Villa is quoted by von Weismayr as having found that guaiacol prevents growth of streptococci in a dilution of 1:1,000, and kills in this dilution in 16 minutes, and in dilution of 1:100 in 2 minutes.

Hammerl<sup>7</sup> found that paracresol is equal to orthocresol in bactericidal power against staphylococci and typhoid bacilli, but more toxic. Phenol was less strongly bactericidal and more toxic than either.

Several authors quote Shaw<sup>8</sup> as having demonstrated that guaiacol is ineffective in infections of animals, but the original article shows that he merely inoculated two rabbits with *B. pyocyaneus*, and injected one with 20 c.c. of a 1:200 guaiacol solution (the lethal dose of which is 25 c.c.). This animal died in 18 hours and the control in 26 hours. There is no other experimental evidence in this much quoted article.

One of the most important contributions to the subject of creosote therapy is that of Bechhold and Ehrlich,<sup>9</sup> who (using especially diphtheria bacilli for their tests) developed many new and fundamental facts in relation to the influence of various modifications of the phenol derivatives on their bactericidal and physiologic action. The chief conclusions were:

1. Introduction of halogens into phenol increases the disinfectant action in proportion to the number of halogen atoms introduced<sup>10</sup> (e. g., 1 mol. pentabrom phenol has the same action on diphtheria bacilli as 500 mol. phenol).

2. Alkyl groups introduced into phenols or halogen phenols increases their disinfectant action. (Tribrom-m-xyleneol is 20 times as active as tribrom phenol; tetrabrom-o-cresol is 16 times as active as tetrachlor phenol).

3. Union of 2 phenols or halogen phenols, either directly or through  $\text{CH}_3$ ,  $\text{CHOH}$ ,  $\text{CHOCH}_3$  or  $\text{CHOC}_2\text{H}_5$  groups, increases activity. Thus, tetrabrom-o-cresol inhibits the growth of diphtheria bacilli in a dilution of 1:200,000, while tetrabrom-o-biphenol inhibits when diluted to 1:640,000.

4. Union of 2 phenols through CO or  $\text{SO}_2$  decreases activity.

5. Introduction of COOH into the nucleus decreases activity.

6. Halogens introduced into phenols at first reduce toxicity, but the trihalogens have about the same toxicity as the unhalogenized substance, and tetra- and penta-halogen compounds are more toxic. However, the spasmodic action of the phenols is reduced in proportion to the number of halogen atoms.  $\text{CH}_3$  groups compensate or neutralize the toxicity introduced by the halogens.

Of the compounds developed in this study the most effective were:

Tetrabrom-o-cresol, which has but little toxicity yet inhibits growth of diphtheria bacilli diluted to 1:200,000, and in 1% solution kills them in less than 2 minutes. It compares in activity with phenol in the ratio of 1,000:0.9.

Tetrabrom-o-biphenol (and the corresponding Cl compounds) which is more toxic but inhibits growth at a dilution of 1:640,000.

Hexabrom-diphenyl carbinol, practically nontoxic, inhibits growth at 1:200,000; kills in 24 hours in dilution of 1:320,000 and kills in 10-15 minutes at

<sup>6</sup> Deut. med. Wehnschr., 1893, 19, p. 781.

<sup>7</sup> Hyg. Rundschau, 1899, 9, p. 1017.

<sup>8</sup> Jour. of Hygiene, 1903, 3, p. 159.

<sup>9</sup> Ztschr. physiol. Chem., 1906, 47, p. 173.

<sup>10</sup> In a Patentschrift, Dammann, in 1889, also mentions this effect of halogens; quoted by Schottelius, Arch. f. Hyg. 1913, 82, p. 76.

1:1,000. Compares with phenol as 1,000 to 0.6 in respect to action on diphtheria bacilli, although less effective against "water bacteria" than phenol.

Although these substances did not precipitate proteins they were ineffective against diphtheria bacilli in serum, and on this basis the authors explain their failure to influence favorably diphtheria infection in animals. Unfortunately, they give no details as to the methods used in conducting these experiments. They merely say "Wir versuchten Hexabromdioxydiphenyl carbinol, Tetra-bromhydrochinonphthalein usw. besonders gegen Diphtherie an Meerschweinchen, Kaninchen, und auch gegen Streptococcen an weissen Mäusen, Tetrabrom-o-Kresol gegen Streptococcen an weissen Mäusen. Der Erfolg blieb aus."

This article was followed by a report by Bechhold<sup>11</sup> under the title of "Halbspezifische chemische Desinfektionsmittel" in which is emphasized the fact that the effect of a given chemical on one species of bacteria may not be duplicated with another species, and hence general laws covering the influence of various modifications of a substance on its bactericidal action cannot be deduced from limited observations. Thus, in the previous article it was stated that the introduction of halogen atoms into phenols increases the disinfectant action somewhat in proportion to the number of added halogens. But Bechhold finds that against staphylococci, streptococci and diphtheria bacilli the maximum disinfectant power is shown by tribrom- $\beta$ -naphthol, as compared with either mono- and di- or tetra- and penta-brom- $\beta$ -naphthol. On the other hand, against paratyphoid bacilli the activity is constant as halogens are added to dibrom or dichlor, and decreases with three or more halogen atoms. The "semi-specificity" of this class of disinfectants is shown by several examples. Thus, tetrabrom-p-biphenol and tribrombikresol are very active disinfectants for staphylococci, but against colon bacilli they are less effective than lysol. While tri- or tetrabrom- $\beta$ -naphthol, tetrabrom-o-cresol and tetrachlor-l-biphenol have a considerable disinfectant action even on anthrax spores, they as well as some others of the higher halogen phenols, are practically inactive against tubercle bacilli. Tetrabrom-o-cresol, hexabromdioxydiphenyl-carbinol, tetrachlor-o-biphenol, tribrom-biphenol and tribromcresol, in 1% solution for 2 hours with an emulsion of human tubercle bacilli did not impair their infectivity for animals. Tri- tetrabrom- $\beta$ -naphthol acted in 2.5% solution on tubercle bacilli for 25 hours without effect, while a 5% lysol solution (containing 2.5% of cresol) kills tubercle bacilli in 4½-8 hours. Hence all these disinfectants which are much more actively destructive of staphylococci than lysol, are much less effective than lysol against tubercle bacilli.

As far as we can learn, the leads given in these articles have not been followed much farther, either in Ehrlich's laboratory or elsewhere. Leubeneheimer<sup>12</sup> has established anew the general applicability of the principle of the effect of halogenized phenols to bacteria, and also demonstrated for different xylenes a high bactericidal action (Schottelius).

Raschig<sup>13</sup> is said to have followed this lead and produced a chlorinated xyleneol which has great bactericidal properties.

Schottelius<sup>14</sup> has investigated the action of "grotan," described as "a complex cresol alkali compound," Na-p-chlor m-cresol. This substance he found to be strongly bactericidal, 0.5% solution killing in 5 minutes all the bacteria

<sup>11</sup> Ztschr. f. Hygiene u. Infektionskr., 1909, 64, p. 113.

<sup>12</sup> Phenol und seine Derivate, Berlin, 1909; quoted by Schottelius.

<sup>13</sup> An incorrect reference to Raschig's work is given and we have not succeeded in locating the correct reference.

<sup>14</sup> München. med. Wchnschr., 1912, 59, p. 2674.



tried (typhoid stools and cultures, staphylococci, streptococci and pus), 0.3% usually being effective, and 0.25% killing in 20-30 minutes; 1% solution killed anthrax spores in 20 minutes. Tuberculous sputum was treated with an equal volume of 2% grotan for 10, 30 and 60 minutes and each injected into guinea-pigs and rabbits without infection resulting, although all the controls were tuberculous after 28 days. No further or more exact tests seem to have been made with tubercle bacilli. The substance is said to be almost nontoxic and nonirritating, 3 gm. subcutaneously not poisoning 4,000 to 6,000 gm. dogs.

He also reports<sup>15</sup> that in a mixture of chlorxylenol and chlorcresol the disinfectant action is not merely the sum of the components, but is increased about 100% above this. Especially efficient is a preparation made by dissolving chlorxylenol in soap and adding to "grotan." A new compound called "sagrotan" is produced in this way, but no exact statement is made as to its preparation or composition, beyond announcing its production by Schülke and Mayr. This substance he found much more strongly bactericidal for anthrax spores, staphylococci, streptococci, typhoid bacilli, and pus or dejecta containing these organisms, than either lysol or liquor cresoli saponatus. Also tuberculous sputum was disinfected by 2% sagrotan in 2 hours, as shown by animal inoculation. It is almost entirely devoid of either local or systemic toxicity. Dogs took 10 gm. per kilo (1% of body weight) into the stomach without serious effects, and Schottelius took 15 gm. at one dose and held his arm 40 minutes in a 10% solution of sagrotan without serious effects. However, 100 gm. in 3 days to a 7.5 kilo dog did not remove intestinal bacteria.

Sagrotan was found by Friedenthal<sup>16</sup> to have an alkalinity, as sold in 10% solution, corresponding to 0.56% KOH, so that injected subcutaneously it causes local necrosis. Guinea-pigs are not affected by subcutaneous injection of 2.5 gm. per kilo. In general, the low toxicity of grotan and sagrotan was corroborated by Friedenthal, who does not discuss their bactericidal effects.

Fehrs,<sup>17</sup> in discussing various preparations of liquor cresoli saponatus, mentions that *Staph. pyogenes aureus* is very susceptible, and *Streptococcus pyogenes* intermediate.

An extensive consideration of the effect of electrolytes in disinfection with cresol soaps is given by Frei,<sup>18</sup> but this contains nothing bearing directly on our problems.

Heukeshoven<sup>19</sup> has made the most extensive study of the action of thiocol, and one of the few studies of the effect of creosote derivatives on tuberculous animals that we can find in the literature. He found that a-thiocol,

  $\text{OCH}_3$   
 $\text{OH}$  and b-thiocol,   $\text{OCH}_3$   
 $\text{SO}_3\text{K}$   $\text{OH}$  were almost absolutely without

inhibiting effect on staphylococci, anthrax and *B. pyocyaneus*, for all these grew in asparagin-glucose agar containing from 1 to 5% of these compounds; while guaiacol carbonate, being used merely as a suspension, had little more effect, but K-guaiacolate prevented growth of staphylococci in 0.5% (the lowest concentration tried), and inhibited anthrax and pyocyaneus at 2% but not at 1%.

The animal experiments were performed with four series of rabbits inoculated with tubercle bacilli (origin not stated) in the eye. In each series were

<sup>15</sup> Arch. f. Hyg., 1914, 82, p. 76.

<sup>16</sup> Berl. kl. Wehnschr., 1915, 39, p. 1019.

<sup>17</sup> Centralbl. f. Bakteriologie, 1. 1904, 37, p. 730.

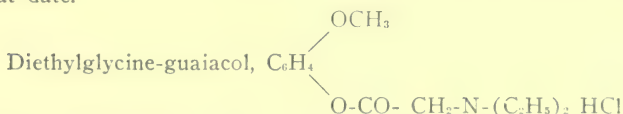
<sup>18</sup> Ztschr. Infektkr. d. Haustiere, 1914, 15, p. 273 and 407.

<sup>19</sup> Experimentelles über die Wirkung des "Thiocols" bei der Tuberkulose, R. Heukeshoven, Inaug. Dissert., Bern, 1899.



nine rabbits, one serving as a control. They received daily doses of 0.5 gm. of each of the 4 guaiacol derivatives mentioned above, by means of a catheter. In one series the drug was given for 14 days before infection and not afterward; in the second the treatment was continued; in the third there was no preliminary treatment, but treatment continued after the infection; in the fourth treatment was not begun until 4 weeks after the inoculation. The result was, in brief, that in the rabbits receiving thiocol-a, 2 recovered completely and in none was the disease disseminated through the body; with thiocol-b, none recovered, in 6 there was no dissemination, and in 2 there was dissemination; with potassium guaiacolate, in 5 of 8 the tuberculosis was disseminated; with guaiacol carbonate it was disseminated in 6 of 8, and in the 4 controls. He also found increase of weight in animals given thiocol-a or guaiacol carbonate, potassium guaiacolate had a deleterious effect on the animals and thiocol-b had no effect. As it has been found that thiocol is excreted unchanged, and as it seems to be devoid of bactericidal properties (although not tested on tubercle bacilli) these favorable effects are difficult to explain.<sup>20</sup>

Lubowski<sup>21</sup> has reviewed the abundant literature on thiocol to the middle of 1904, but this literature contains no work that seems to be accurate except that of Heukeskovén, nor have we found any contributions of importance since that date.



known commercially under the name Guiasanol, has been produced and described by Einhorn<sup>22</sup> as having many therapeutic advantages. Gastric irritation is avoided because the free base is not formed until the HCl is split off in the intestinal alkalies, and free guaiacol is eventually liberated since it is found in the urine.<sup>23</sup> The toxicity is low, rabbits being uninjured by 2 gm. subcutaneously, or 4 gm. by stomach, and 2% solutions are not irritating. Buchner tested the bactericidal action of guiasanol, finding it low in vitro, for it inhibited the growth of *B. coli*, *B. pyocyaneus*, *B. proteus* and *S. pyogenes aureus* only in concentrations of 1:50 or 1:100. If it is correct that guiasanol readily liberates guaiacol in the body, the low bactericidal power in vitro is of little significance, and Einhorn claims that it is efficient as a local antiseptic in ulcerating cancer and tuberculous enteritis.

A few other incidental references complete the list.

Burrow<sup>24</sup> reported that a 3% aqueous solution of sodium or potassium guaiacolate containing 0.01% potassium arsenite prevented growth of tubercle bacilli, although without the arsenic the guaiacol was ineffective. He also stated that the blood of a rabbit treated with this mixture would not support growth of tubercle bacilli, and that guinea-pigs and rabbits thus treated resisted tuberculous infection. These results he attributed chiefly to the arsenic. This

<sup>20</sup> Lyngkol, which differs from Thiocol in having  $\text{C}_6\text{H}_4$  instead of  $\text{K}$ , is said by Takenaka (Japanese Jour. Tuberc. Kekkaku Zasshi, 1919, 7, No. 1) to be as strongly bactericidal for tubercle bacilli as creosote and guaiacol, which all, he says, inhibit growth at concentrations over 1:10,000.

<sup>21</sup> Allg. Med. Centr. Ztg., 1905, 74, pp. 337, 336, 396.

<sup>22</sup> Münch. med. Wchnschr., 1900, 37, p. 40.

<sup>23</sup> Concerning absorption and elimination of guaiacol see Fischele, Ztschr. klin. Med., 1896, 29, p. 197.

<sup>24</sup> Münch. med. Wchnschr., 1910, 37, p. 1391.

<sup>25</sup> Münch. med. Wchnschr., 1911, 38, p. 1007.

work, the report of which does not give a convincing impression, was repeated by Nürnberger,<sup>25</sup> who found no effects produced in either cultures or animals by the guaiacol-arsenic mixture of Burów, or by 0.01 gm. Na guaiacolate in glycerin-agar, the concentration not being stated.

J. Naberly<sup>26</sup> reported favorable clinical effects with a "new guaiacol chlor-iodid compound," the exact nature of which is not given. As there is no experimental evidence in regard to this compound, it is mentioned only because it has some possible relation to Ehrlich's observations. The Lancet laboratory examined this compound and found the iodine and chlorine nearly all in chemical union.

Cooper<sup>27</sup> reports an extensive study of creosote and allied substances, particularly with reference to the disinfectant action of various soap solutions in surgery and disinfection work. The chief facts developed of interest in this connection are:

Using the Rideal-Walker method as modified by Chick and Martin, the phenol coefficient of the cresols in pure aqueous solution was found to be:

	<i>B. typhosus</i>	<i>S. pyogenes</i>
Ortho cresol .....	2.6	2.1
Meta cresol .....	2.6	2.0
Para cresol .....	2.6	2.4
Thymol .....	25.0	...
"Cresylic acid" .....	...	2.2

Therefore, as shown by thymol,  $\text{C}_6\text{H}_4\text{C} \begin{array}{c} \text{HC} \quad \text{CH} \\ \diagup \quad \diagdown \\ \text{HO-C} \quad \text{CH} \end{array} \text{C-CH}_3$ , alkyl groups in the

benzene ring may increase greatly the germicidal action, which accords with Bechhold's and Ehrlich's statements, and with the observation made by Koch in 1881.

On the other hand, the introduction of a second OH group into phenol decreases bactericidal action. Thus, for typhoid bacilli in water the phenol coefficient of various OH compounds was: resorcin, 0.3; pyrocatechin, 0.5; hydroquinone, 1.0; pyrogallol, 0.77; phloroglucin, less than 0.35. Quinone,  $\text{O} \begin{array}{c} \diagup \quad \diagdown \\ \text{O} \end{array}$ , however, had a phenol coefficient for staphylococci of 10, whereas acetone was less than 0.075.

Krauss,<sup>28</sup> in describing several new compounds of trypan red, gives the method of making guaiacol trypan red and iodo-guaiacol trypan red. He says nothing concerning their activity, but Paul Lewis in a brief note published elsewhere<sup>29</sup> states that no effects had been obtained in experimental tuberculosis with any of their trypan red compounds.

#### BACTERICIDAL AND BACTERIOSTATIC EXPERIMENTS

In order to determine whether there is any reason for believing that the various members of the guaiacol series should be expected to have any direct action on tuberculosis, the inhibitory or bacteriostatic action

<sup>25</sup> Lancet, 1913, 2, p. 285.

<sup>27</sup> Brit. Med. Jour., 1912, 1, p. 1234.

<sup>26</sup> Jour. Amer. Chem. Soc., 1914, 36, p. 960.

<sup>29</sup> Jour. Pharm. and Exper. Therap., 1914, 4, p. 353.

of a considerable number of the series has been tested on the bacillus of human tuberculosis. Several strains of the organism were used; in some cases no note as to the strain was made. In most of the tests, a strain which had been growing in our laboratory for a number of years and which we have distinguished from other strains acquired more recently, by the name "old human" was used. This strain has diminished somewhat in virulence but has not changed its growth characteristics. We use the following method for testing inhibitory power: To tubes containing a certain definite amount of glycerol agar, the chemical to be tested is added in sufficient quantity to make the desired dilutions. The dilutions range from 10% up to 0.0001%. Two of the substances used (styracol and guaiacol cacodylate) are quite insoluble in water. To make dilutions of these, the required amount of dry powder was added to hot agar, well shaken, and the agar cooled and slanted quickly before the powder settled out. Table 1 gives a summary of the experiments on the inhibitory action of all the tested drugs of this series.<sup>30</sup>

From this table, it may be seen that 0.01% or 1 in 10,000 dilution completely inhibited the growth of the tubercle bacillus in the test with resorcin, thymol, p-cresol, m-cresol and o-cresol; that 0.05% was the lowest concentration which completely inhibited in the case of creosol and pyrocatechin; guaiacol, creosote, hydroquinone and guaiacol cacodylate required a concentration of 0.1% or 1 in 1,000 to inhibit growth completely. Sodium guaiacolate inhibited completely at 1.7% and partially at 0.8%. Thiocol and styracol caused no inhibition at 1% concentration while the organisms grew well even in a suspension of 10% of styracol, which seems to be almost completely insoluble in water.

The bactericidal power of many of these compounds was then tested in the following way. The "old human" strain was used in all the tests. Six dilutions of the chemicals to be tested, from 1 in 100 to 1 in 1,000,000, were made in water. Small clumps of cultures were then immersed in these solutions, remaining in them 10 minutes, 1 hour, 6 hours, 24 hours and 48 hours. At the end of the desired time, the clump was removed, washed in several waters to remove the chemical and finally planted in agar tubes. Controls were made by exposing the clumps to normal salt solution for the same periods of time and then washing them in the same way. They all grew luxuriantly.

<sup>30</sup> Some of these experiments were carried out by Dr. Radha D. Dhill.



The tubes inoculated with the clumps which had been exposed to 1 per cent. orthocresol for one hour developed a slight growth, while the 6 hour, 24 and 48 hour sets showed no growth. One per cent. meta-cresol, paracresol and thymol killed all the cultures after one hour's exposure, while 1 per cent. thymol killed all the organisms in 10 minutes. There was but little growth in the tubes inoculated with the clumps exposed for 10 minutes to 1 per cent. metacresol and paracresol. Creosol, resorcin, hydroquinone and pyrocatechin had no bactericidal effect in this experiment even in 1 per cent. concentration for 48 hours

TABLE 1  
INHIBITION OF GROWTH OF HUMAN TUBERCLE BACILLI

Per- cent- age	Creosote			Guaiaacol			O-cresol			M-cresol			P-cresol			Thymol			Creosol			Resorcin		
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.8																								
0.5										—	—	—	—	—	—				—	—	—			
0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.05							—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
0.01	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	++	++	++	—	—	—
0.001	++	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+++	+++	+++	+	+	+
0.0001	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+++	+++	+++	+	+	—

Blank spaces indicate that that dilution was not used.  
The — sign shows that there was no growth (complete inhibition).

and concentrations below 1 per cent. had no bactericidal effect with any of the drugs, even 0.1 per cent. thymol failing to kill in 48 hours, although 1 per cent. killed completely in 10 minutes.

Creosote and guaiaacol were also tested by the same method but different dilutions were used. One hundred per cent. of either creosote or guaiaacol killed all the organisms in 5 minutes so that there was no growth on the agar tubes. Five per cent. dilution killed some in 5 minutes and killed all in 1 hour; 1% creosote killed all in 1 hour and in 6 hours, but it required 6 hours for complete tuberculocidal action of 1% dilution of guaiaacol. There was no growth in tubes after one hour's exposure to 0.5% dilution of creosote and 6 hours and 24 hours exposure to 0.1% creosote killed some of the organisms. Guaiaacol, on the other hand, showed little bactericidal action in concentrations lower than 1%.

From these results, it may be seen that the bactericidal power of these substances is much lower than the bacteriostatic power. One per cent. was the only concentration which had any marked bactericidal power as shown by this method; 1% thymol killed all the organisms even in 10 minutes, so that they failed to grow when planted on agar tubes; 1% of metacresol and paracresol killed in 1 hour and of orthocresol in 6 hours. Even 1% of creosol, resorcin, hydroquinone or pyrocatechin failed to kill. One per cent. creosote killed  $K_4$  bacillus in one hour and the Y. Miller strain was killed in one hour by 0.5% of creo-

TABLE 1—*Continued*  
INHIBITION OF GROWTH OF HUMAN TUBERCLE BACILLI

Thiocol			Styracol			Sodium Guaiacolate			Guaiacol Carodylate		Hydroquinone		Pyrocatechin	
+++	+++	+++	+++	+++	+++	---	---	---	---	---	---	---	---	---
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
+++	+++	+++	+++	+++	+++	---	---	---	---	---	---	---	---	---
+++	+++	+++	---	---	---	+	+	+	---	---	---	---	---	---
+++	+++	+++	+++	+++	+++	---	---	---	+	---	---	---	---	---
+++	+++	+++	+++	+++	+++	+	+	+	+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	---	---	---	---	---	---	---	---	---
+++	+++	+++	+++	+++	+++	---	---	---	---	---	---	---	---	---

The + sign shows growth, +++ more luxuriant growth and ++++ very luxuriant growth. Controls were grown in all cases and always showed very luxuriant growth.

sote. It required 5% guaiacol to kill the "old human" in 1 hour although 1% killed all the organisms in 6 hours.

We realized that the clump and test tube method of bactericidal work could not be considered absolutely reliable, since some of the clumps may not be completely permeated by the chemical while others will be saturated and broken up so that they are difficult to transplant to new mediums. For these and other reasons, the results as given above are not entirely consistent. Therefore it seemed best to repeat the bactericidal experiments with some, at least, of the compounds, using more delicate methods for determining the results. We chose the so-called garnet method, which we used thus:

1. A sufficient number of garnets of fairly uniform size to allow ten for each animal, were thoroughly cleaned with sulphuric acid, water, acetic acid, water, alcohol and finally ether. They were well

dried and sterilized. A suspension of tubercle bacilli was then made in which the garnets were well shaken and allowed to stand for a time. The fluid was decanted off and the garnets dried over sterile calcium chlorid. When dry, the required number of garnets were placed on perforated platinum baskets and immersed in about 50 c c of the various dilutions for the desired periods of time. The dilutions used were 1%, 0.5%, 0.1% and 0.01% and the periods of exposure were 20 minutes, 1 hour, 6 hours and 24 hours. Controls were exposed to physiological sodium chlorid solution for the same periods of

TABLE 2  
ANIMAL TEST OF BACTERICIDAL ACTION

Dilutions	Duration of Exposure	Creosote						Gualacol					
		I		II		III		I		II		III	
		Local	General	Local	General	Local	General	Local	General	Local	General	Local	General
1%	20 minutes.....	+	—	—	—	+	—	—	—	+	—	+	—
	1 hour.....	+	—	—	—	—	—	+	—	+	—	+	—
	6 hours.....	+	—	+	+	—	—	+	—	—	—	+	—
	24 hours.....	—	—	—	—	—	—	+	—	+	—	+	—
0.5%	20 minutes.....	+	—	+	—	—	—	+	—	+	—	+	—
	1 hour.....	+	—	—	—	—	—	+	—	—	—	—	—
	6 hours.....	—	—	—	—	—	—	+	—	—	—	+	—
	24 hours.....	—	—	+	—	—	+	—	—	+	—	—	—
0.1%	20 minutes.....	+	+	+	+	+	—	+	+	+	+	—	—
	1 hour.....	+	+	+	+	+	—	+	+	+	+	—	+
	6 hours.....	+	+	+	+	+	+	+	+	+	+	+	+
	24 hours.....	+	+	+	+	—	+	+	+	+	+	+	+
0.01%	20 minutes.....	+	+	+	+	+	—	+	+	+	—	+	+
	1 hour.....	+	+	+	+	+	+	+	+	+	+	+	+
	6 hours.....	+	+	+	+	+	+	+	+	+	+	+	+
	24 hours.....	+	+	+	+	+	+	+	+	—	+	+	+

Controls were inoculated with cultures which had been exposed to physiologic salt solution for the same periods of time and otherwise treated in the same way as the medicated cultures. All the control animals exhibited tuberculous lesions, either local or general or both.

time and then treated in the same way. At the end of the exposure, the basket with the garnets was lifted out and washed through three dishes of water and one of physiological salt solution. Then the garnets were transferred into sterile test tubes, each containing 2 c c of sterile physiological sodium chlorid solution, 10 garnets being counted into each tube and 3 or 4 tubes being allowed for each set. These tubes were then shaken thoroughly for one-half hour in a shaking machine to remove the bacteria from the garnets, and the salt solution was then injected subcutaneously into guinea-pigs, the entire amount in one tube being used for one pig. Therefore, there were 3 guinea-pigs in each set, or 60 for each experiment. In this method, the bacteria are in a



thin layer on the garnets so that there is no question of permeation of the layer. There is much less danger of contamination from handling and the guinea-pig response to inoculation is much more delicate than that of the agar tube. Table 2 gives a summary of the results of these experiments.

From this table, it may be seen that 1% was bactericidal in all the compounds except resorcin and even resorcin had in that concentration some bactericidal power, especially if the exposure lasted 24 hours. Creosote and guaiacol had marked bactericidal power in 0.5% concen-

TABLE 2—Continued

ANIMAL TEST OF BACTERICIDAL ACTION

[illegible]

tration and thymol killed practically all the organisms in 0.1% dilution. The + sign in the tables indicates that the bacteria developed either in the test tube or in the animal body. If, in table 2, the development of the tuberculous process occurred only in the lymph glands near the point of inoculation, the + sign is under "local." If other glands or other organs in the body are involved, the sign is placed in the column marked "general." It may be noted that results are not entirely consistent even by this method and that the — sign occasionally occurs where we have every reason to expect the + sign, even in the controls. This is probably due to the fact that in the shaking machine some of the tubes may be so placed that the garnets are not shaken violently enough to release a sufficient number of bacteria to cause an infection.

It may in some cases be due to the fact that some of the garnets are not well dried so that the bacteria are washed off before ready for shaking and hence are lost in the germicide or in the wash waters. The results are, however, sufficiently consistent to give us a definite idea of the limit of bactericidal action.

#### THERAPEUTIC EXPERIMENTS

*Creosote*.—Tests on toxicity of creosote were made in order to determine a safe dosage:

One guinea-pig received an intracardiac injection of 3 cc of  $\frac{1}{200}$  creosote in physiological salt solution or 0.015 cc creosote with no ill effects.

One guinea-pig received an intrapéritoneal injection of the same amount, 0.015 cc, with no ill effects.

TABLE 3  
THERAPEUTIC TEST OF CREOSOTE (SET 1)

Duration of Treatment	Total Amount Creosote Received	Loebl Glands	Liver	Spleen	Lungs	Internal Glands
90 days	Fed 0.462 cc Injected 0.067 $\frac{1}{2}$ cc	—	—	—	—	—
204 days	Fed 1.05 cc Injected 0.067 $\frac{1}{2}$ cc	—	+ ?	—	—	—
298 days	Fed 1.506 cc Injected 0.067 $\frac{1}{2}$ cc	—	—	—	—	—
380 days	Fed 1.44 cc Injected 0.067 cc	+++	+++	—	—	++++
180 days	Fed 0.924 cc Injected 0.067 cc	++	+++	+++	+++	++
112 days	Fed 0.546 cc Injected 0.067 cc	+++	+++	+++	—	+++
141 days	Fed 0.776 cc Injected 0.067 cc	+++	+++	++++	—	+++

One guinea-pig received a subcutaneous injection of 0.1 cc creosote in physiological salt solution with no ill effects and no ulceration of the skin.

*Series 1*.—These 3 pigs and 6 normal pigs were then inoculated with 0.2 cc dilute emulsion of "old human" tuberculosis.<sup>31</sup> These 9 pigs were fed daily, except Sunday, one pill containing 0.003 cc creosote. Each received in addition one intramuscular injection of 0.005 cc creosote in 1 cc cotton seed oil, one intracardiac injection of 0.01 cc creosote in 2 cc physiological salt solution, 5 subcutaneous injections of 0.005 cc in 1 cc physiological salt solution and 3 subcutaneous injections of 0.0075 cc each in 1 cc physiological salt solution. The injections were made once a week for 10 weeks and the feeding was continued until death. Two of the pigs died immediately after the intracardiac injection, too early to show much general involvement, although the inguinal glands were enlarged and caseous. Table 3 gives results in the other 7 animals.

<sup>31</sup> In all these experiments, the animals of series 1 were inoculated with an amount of culture determined, not by weighing, but by the opacity of the suspension. Sufficient sodium chlorid solution was added to make the suspension slightly opalescent. In the animals of series 2, the amount was determined by weight.

*Series 2.*—In this series, 9 guinea-pigs were inoculated subcutaneously with 0.2 mg. of Corper's 1305<sup>32</sup> in 0.2 c.c. of physiological salt solution. These were fed daily 0.2 drop creosote in 2 drops cotton seed oil. Five subcutaneous injections, one each week, were given of 2 drops creosote in 1 c.c. physiological salt solution.

Table 4 gives the results.

TABLE 4  
THERAPEUTIC TEST OF CREOSOTE (SET 2)

Duration of Treatment	Total Amount Creosote Received	Local Glands	Liver	Spleen	Lungs	Internal Glands
84 days	Fed 14.22 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
105 days	Fed 18.0 drops					
	Injected 9.0 drops	+++	+	+++	+++	+++
123 days	Fed 21.2 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
126 days	Fed 21.6 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
126 days	Fed 21.6 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
128 days	Fed 22.0 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
133 days	Fed 22.8 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
152 days	Fed 26.0 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
154 days	Fed 26.4 drops					
	Injected 9.0 drops	+++	+++	+++	+++	+++
220 days	Fed 37.8 drops					
	Injected 9.0 drops	+	+++	+++	+++	+++

Four of this set had definite and some fairly large cavities in the lungs an unusual proportion.

Table 5 gives for comparison the conditions in untreated control guinea-pigs inoculated with the same dose of the same two strains of tubercle bacilli. It may be noted that some of the controls of set 1 failed to develop the disease even after 334 days. In other words, the treatment seems, with creosote, as well as with many other drugs, to lower the resistance of the animal to the disease.

TABLE 5  
CONTROLS OF SET 1 OF THIS SERIES

Duration of Treatment	Local Glands	Liver	Spleen	Lungs	Internal Glands
64 days.....	+	—	—	—	—
96 days.....	—	—	—	—	—
97 days.....	+	—	—	—	—
287 days.....	+	++	+++	+++	+++
334 days.....	—	—	—	—	—
461 days.....	—	—	—	—	—

*Guaiacol Treatment Experiments.*—Dec. 15, 1916, to determine the safe dose of guaiacol, 3 guinea-pigs were injected with guaiacol, 1:200 of physiological salt solution. One received 3 c.c. by intracardiac injection, one 3 c.c. by intra-

<sup>32</sup> This culture was isolated by Dr. Corper from the sputum of a tuberculous patient at the Chicago Municipal Tuberculosis Sanatorium about 1913, and has been grown in this laboratory ever since; 0.05 mg. injected subcutaneously into guinea-pigs, causes in every case generalized tuberculosis with death in from 4 to 6 months.



peritoneal injection and one received 2 cc by subcutaneous injection. As there were no ill effects except slight infiltration and induration of tissue surrounding the point of subcutaneous injection, the 3 were inoculated with 0.2 cc of a dilute suspension of "old human" tubercle bacilli and 6 other pigs received the same inoculation. Then each of the 9 received daily by mouth a pill containing 0.003 cc guaiacol and also an injection weekly. One injection was intramuscular, one was intracardiac, and seven were subcutaneous. One of these pigs died after 20 days, showing only involvement of a regional lymph gland, while 1 lived 929 days and exhibited at death no tuberculous involvement either local or general. Only 5 of the 9 guinea-pigs in this series showed advanced general tuberculosis; this corresponded fairly well with the controls of the same series in which only 2 of the 6 infected animals showed a general tuberculosis.

*Guaiacol Treatment.*—Set 2. In this set, Corper's 1305 was used and 0.2 mg. was injected subcutaneously. Ten guinea-pigs were inoculated at the same time. They were fed daily at first 0.2 drop in 2 drops cotton seed oil, then 0.3 drop and later 0.5 drop. Weekly subcutaneous injections of guaiacol were given in physiological salt solution, 1 drop at first, quickly increased to 2 drops in 1 cc of physiological salt solution. Of this set, one died in 21 days, having no tuberculous involvement. All the others exhibited a marked general tuberculosis of lymph glands, liver, spleen and lungs, the degree of involvement being about the same as that shown in the control animals of series 2, summarized in table 6. The last one of the set to die lived 191 days and had received a total of 90 drops of guaiacol.

TABLE 6  
CONTROLS FOR SET 2 OF ALL THESE EXPERIMENTS

Duration of Disease	Local Glands	Liver	Spleen	Lungs	Internal Glands
84 days.....	+++	++	++	+	—
98 days.....	+++	+++	+++	+++	+++
107 days.....	+++	+	+	+	+++
107 days.....	+++	+++	+++	+++	+++
114 days.....	+++	+++	+++	+++	+++
122 days.....	+++	++++	++++	++++	+++
138 days.....	+++	++++	++++	++++	+++
159 days.....	+++	+++	++++	++++	+++
163 days.....	+++	++++	+++	+++	+++
223 days.....	+++	++++	+++	+++	+++

Two of the animals had lungs containing cavities.

*Creosol Treatment Experiments.*—The first set consisted of 9 guinea-pigs, 3 of which had been previously used to determine the safe dose of creosol, and all were inoculated in January, 1917, with 0.2 cc of dilute emulsion of "old human." Four died or were killed too early to show any development of the disease. Two of these died immediately after an intracardiac injection, one from pneumonia and one from being crushed in the cage. In 2 of these early deaths, in which the guinea-pigs had lived 2 weeks, the animals showed early signs of tuberculosis, such as enlarged glands, and one had a few young tubercles in the lungs. The others were fed daily 0.2, 0.3 and 0.5 drop of creosol in cotton seed oil up to the time of death, and received 11 subcutaneous injections of creosol in physi-

ological salt solution at weekly intervals. Four of the 5 remaining animals in this set showed generalized tuberculosis, a much larger proportion than was seen in the untreated controls of the same set.

*Creosol Treatment.*—Set 2 was inoculated Feb. 4, 1918, with 0.2 mg. of culture 1305 and fed daily 0.2 of a drop for 12 days, 0.3 of a drop, then the rest of life 0.5 of a drop in cotton seed oily daily, except Sundays. The animals were injected weekly for 6 weeks with 2 drops in physiological salt solution. Ten guinea-pigs were used in this series. The first to die lived 80 days and the last 212 days; all showed a marked generalized tuberculosis, corresponding to the untreated controls of the same series (see table 6).

*Thiocol Treatment.*—Series 1: Jan. 3, 1917, six pigs were inoculated subcutaneously with 0.2 cc weak suspension of "old human" culture. From Jan. 4 on, they were fed daily one 5 mg. pill. After Jan. 18, they were fed two 5 mg. thiocol pills daily. On Jan. 10 and each week thereafter they were injected subcutaneously 5 mg. in 1 cc physiological salt solution the first 3 weeks and afterward 10 mg. in 1 cc physiological salt solution. The thiocol was easily soluble and seemed to have no ill effects. Four of the 6 animals of this set showed marked generalized tuberculosis.

*Thiocol Treatment.*—Series 2: Ten guinea-pigs were inoculated Feb. 4, 1918, subcutaneously with 0.2 mg. of Corper's 1305. From Feb. 5 to March 14, they were fed daily 5 mg. of thiocol and after that, 10 mg. Once a week, they were injected subcutaneously, 5 mg each time the first two weeks and 10 mg. each time thereafter. One of the pigs in this set died in 50 days; the last one died after 287 days, having received 2350 mg. of thiocol. All showed marked generalized tuberculosis, and no effect from the treatment.

*Styracol Treatment.*—Set 1: Jan. 3, 1917, the guinea-pigs were inoculated with 0.2 cc of "old human." Styracol is insoluble in water and the injections were therefore made in cotton seed oil, some intramuscular and some subcutaneous. These injections were made once a week and pills were fed daily except Sunday, 5 mg. for the first 2 weeks and 10 mg. from then on. The only effect of this treatment seen was an increase of generalized tuberculosis over the controls, since all 6 treated animals developed marked generalized tuberculosis while only 2 of the 6 controls showed any involvement more than a slight enlargement of the regional lymph glands.

*Styracol.*—Set 2: Ten guinea-pigs were inoculated with 0.2 mg. 1305. Treatment was the same as in set 1. One of the 10 guinea-pigs of this set died in 42 days with no tuberculous involvement except in the regional lymph glands, all the other pigs of this set, the last of which lived 247 days and received 1950 mg. of styracol, showed marked generalized tuberculosis, corresponding to controls given in table 6.

*Orthocresol, Metacresol, Paracresol and Thymol.*—Treatment: The guinea-pigs received inoculations with 0.2 mg. of culture 1305. They were fed daily 0.001 cc of the indicated drug in pill form. After the first 3 weeks, they were fed 0.002 cc daily. They also received subcutaneous injections once a week, usually 0.003 cc in water, but part of the time 0.006 cc was injected. The weekly injections and daily feedings were kept up until death. Thymol, being a solid, was weighed and 1 or 2 mg. were fed daily and 3 or 6 mg. were injected. Of the animals treated with these four drugs, none showed any effect of treatment, if we except a slight diminution of tuberculous involvement in pigs treated with paracresol and thymol. The first of the paracresol set to die lived 120 days and showed practically no generalized tuberculosis, while

even one dying after 134 days showed only a few small tubercles in the lungs, liver and spleen. The ones dying later, however, had advanced involvement of all the internal organs which are subject to tuberculous infection. Of the thymol set, 1 died in 58 days, 1 in 80 days and a third after 115 days with no or slight general involvement and 1 animal is still living and apparently well after 239 days. However, 1 dying after 136 days and 1 after 147 days showed marked generalized tuberculosis. The controls and the animals treated with orthocresol and metacresol showed without exception advanced tuberculosis, even in those dying at 73 and 87 days. The longest period of life of the controls was 171 days, of the metacresol treated, 107 days and of the orthocresol treated, 188 days.

All of the compounds of this series have shown so little local toxicity that it was at no time necessary to stop the injections on account of infiltrations, necrosis and ulcerations of the skin, and so little general toxicity that neither the weights, which were taken weekly as a guide to treatment, nor the general condition of the animals ever suggested the desirability of stopping either the feeding or the injections. Twelve normal guinea-pigs have been treated for 125 days with thymol and the cresols, using 3 animals for each drug. The same doses and method of administration have been used with these as with the tuberculous guinea-pigs. Two of these nontuberculous pigs died early from acute cage infections, but the rest are living, in good condition and gaining weight. Hence we may say that all of these drugs are relatively innocuous to guinea-pigs. We have not used intravenous injections in our experiments, since frequent intravenous injections into the same guinea-pig are difficult to make and the intra-cardiac injections are dangerous.

TABLE 7  
AMOUNT OF ORGAN INVOLVEMENT IN TREATED TUBERCULOUS GUINEA-PIGS

Control	Creosote	Gualaccol	Creosol	Thio-col	Styracol	Orthocresol	Metacresol	Paracresol	Thymol
40 97 98%	55 5/7 98.5 .....	66 1/9 88 .....	68 94 .....	66% 89.3 .....	85 87 .....	94	91%	78.6	62

In estimating the therapeutic effect of drugs, they must be judged in several ways. In human patients, it is usual to say that a drug checks or lessens cough, increases or diminishes expectoration, lessens pain and relieves other symptoms. Since experimental animals exhibit few, if any, of these symptoms, we cannot judge in this way. At the death of the animal, we can determine whether the disease is present or absent in the organs, and, if present, how its degree compares with that in control animals which died at approximately the same time after inoculation. We can also compare the duration of the disease in the treated animals with that in the control animals. In order to compare averages of these drugs with reference to the extent of the disease after their therapeutic use, we have endeavored in table 7 to represent the degree of the disease in terms of percentage, calling an extreme



involvement of all the organs 100% and so on down to 0 where all were — and then averaging these percentages.

It may be seen from table 7 that in the first set, which represented the less virulent strain of tubercle bacilli, the degree of the disease was much greater in the treated animals than in the controls, suggesting a possible stimulation of the growth or reduction in resistance. In the second set, the degree of the disease averaged less in the treated animals than in the controls, except in the case of creosote, while in the third set, the degree of the disease was much less in the treated animals than in the controls. A part of this difference may be due to earlier death of the treated animals, since the duration of life may influence the extent of the disease.

The prolongation of life, if at all marked and consistent, may also indicate some beneficial influence of a drug. For these reasons, it seems best, for purposes of comparison, to insert table 8 giving the average duration of life.

TABLE 8  
DAYS OF DURATION OF LIFE OF TREATED TUBERCULOUS GUINEA-PIGS

Control	Creosote	Guaia-col	Creosol	Thio-col	Styra-col	Ortho-cresol	Meta-cresol	Para-cresol	Thy-mol
223.2	185.4/7	285.0	134.2	146.0	150.3				
131.5	135.1	115.3	139.0	147.6	123.9				
157.5	.....	.....	.....	.....	.....	114.6	86.5	140	107.2

From all these facts, we must conclude that none of the compounds belonging to the guaiacol series, so far as we have tested them, has shown definite therapeutic action in experimental tuberculosis in guinea-pigs.

#### SUMMARY

Despite the extensive use of creosote and related compounds in the treatment of tuberculosis, practically no evidence exists as to the susceptibility of *B. tuberculosis* to antiseptics of this class, or as to their influence on the course of tuberculosis in experimental animals. A study of these problems showed that:

Virulent human tubercle bacilli are inhibited from growth (bacteriostatic action) on artificial mediums containing a concentration of 0.01% or 1 part in 10,000, each of resorcin, thymol, paracresol, orthocresol and metacresol; 0.05% (1:2,000) is the lowest concentration which completely inhibited in the case of creosol and pyrocatechin. Guaiacol, creosote, hydroquinone and guaiacol cacodylate required a concentration of 0.1% (1:1,000) to inhibit growth completely.

Sodium guaiacolate inhibited completely at 1.7%, and partially at 0.8%. Thiocol did not inhibit in 1% concentration and styracol, which is insoluble, did not inhibit in 10% concentration (suspension).

Bactericidal tests, in which the capacity to grow on agar after exposure of clumps of tubercle bacilli to the antiseptic was the measure of action, showed that the bactericidal power of these substances is low. Exposure to even 1% solutions of pyrocatechin, hydroquinone, resorcin, and 0.5% solution of creosol, for periods from 10 minutes to 48 hours, entirely fails to kill human tubercle bacilli. Metacresol and paracresol kill in 1% concentrations after exposure for one hour, but not after 10 minutes, while orthocresol reduces growth after 1 hour, and kills in 6 hours. Thymol kills even in 10 minutes at 1% concentration, but 0.1% concentration does not kill even in 48 hours. Weaker concentrations of all these antiseptics were, of course, without bactericidal effect.

A bactericidal test was made with the tubercle bacilli exposed to the antiseptics when in a thin layer on the surface of garnets, and viability determined by inoculating guinea-pigs with the treated bacilli washed from the garnets. Resorcin in 1% solution killed the tubercle bacilli only after 24 hours' exposure. Orthocresol killed in 1% concentration even in 20 minutes, but 0.5% did not kill even in 24 hours. Creosote and guaiacol both killed most of the bacilli in 0.5% concentration, even in 20 minute exposure, but 0.1% concentration was not bactericidal in 24 hours. Thymol was bactericidal in 0.1% concentration, even in an exposure of 20 minutes, but 0.01% was not bactericidal in an exposure of 24 hours.

Therapeutic tests were made on guinea-pigs injected subcutaneously with two strains of human tubercle bacilli, one highly virulent and the other much less so. The animals were then given several doses of the drug by the intracardiac, intramuscular and subcutaneous routes, and daily feedings of pills containing the drugs, the following being tested: creosote, guaiacol, creosol, thiocol, styracol, orthocresol, metacresol, paracresol and thymol. In all, 106 guinea-pigs were thus treated for long enough periods to observe the results (besides the control experiments). Apparently the animals injected with the less virulent tubercle bacilli showed more active tuberculosis than the controls, as if the treatment had lowered their resistance or stimulated the bacilli. With the more virulent bacilli the extent of the disease was perhaps slightly less in the treated animals, probably because they commonly died a little sooner than the controls.

Our experiments show that substances of the creosote series do not possess a high bactericidal power for the tubercle bacillus in vitro, and apparently not in vivo. This result is not surprising in view of the observations of DeWitt and Sherman<sup>30</sup> that tubercle bacilli are rather less susceptible to fat-soluble, and more susceptible to water-soluble antiseptics, than bacteria less rich in fat than the tubercle bacillus. Also by their observation that fat-soluble dyes do not readily penetrate tubercle bacilli, while certain fat-insoluble dyes (e. g., methylene blue) stain them well. Apparently the lipin-rich character of the tubercle bacilli does not make them vulnerable to fat-soluble antiseptics, but rather the reverse.

The figures given above for the bactericidal power may be compared with those obtained by DeWitt and Sherman, using similar methods. Phenol kills in 1% concentration, and shows some effect in 0.1% concentration. Formaldehyd kills in 1% in 1 hour, in 0.1% in 24 hours. Ethyl alcohol, 25%, kills in 1 hour or less. Acetone, chloroform and ether have little or no tuberculocidal action; toluene and iodine show slight influence. Mercuric chlorid kills in 0.001% in 24 hours, 0.1% in 1 hour; gold chlorid in 0.005% kills in 24 hours, as do 0.25% silver nitrate, 0.1% gold tricyanid and 5% copper chlorid. Evidently creosote, guaiacol and the cresols, have about the same tuberculocidal power as phenol, which is distinctly not high. The dihydroxy phenols, resorcin, hydroquinone and pyrocatechin, seem to be less active than the monhydroxy phenol. Thymol was, in all experiments, distinctly, although only slightly, more bactericidal than the other substances tested. This agrees with the statement of Bechhold and Ehrlich<sup>9</sup> that addition of alkyl groups to phenols increases their disinfectant action.

The failure to observe any beneficial therapeutic effect on tuberculous guinea-pigs is, in view of the low bactericidal power of the substances tested, to be expected. It does not mean, however, that these substances may not have value in open tuberculous infections in man in which other bacteria than *B. tuberculosis* are involved. But it does substantiate the opinion that seems to have been generally reached by careful clinical observers, that creosote and guaiacol do not have a specific action on tuberculous infection.

<sup>30</sup> Jour. Infect. Dis., 1914, 15, p. 245.





# **Action of Mercurochrome-220 and of Mercurophen**

A Preliminary Report of Effects on the Human Tubercle  
Bacillus and on Experimental Tuberculosis  
in Guinea-Pigs

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LYDIA M. DEWITT, B.S., M.A., M.D.  
CHICAGO





# ACTION OF MERCUROCHROME-220 AND OF MERCUROPHEN

A PRELIMINARY REPORT OF EFFECTS ON THE  
HUMAN TUBERCLE BACILLUS AND ON  
EXPERIMENTAL TUBERCULOSIS IN  
GUINEA-PIGS \*

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Mercurochrome-220, prepared by Young, White and Schwartz, and mercurophen, made by Schamberg, Kolmer and Raiziss, are two organic mercurial preparations that have attracted unusual attention because of their high germicidal activity, their low toxicity, and their slight local irritating action. Young, White and Schwartz<sup>1</sup> showed that mercurochrome-220, which is a mercury compound of fluorescein, has a high and rapid bactericidal action in urine or hydrocele fluid on most of the ordinary bacteria, and that a 1 per cent. solution could be injected into the urinary bladder and retained for some time. Clapp and Martin<sup>2</sup> reported favorable results from its use in gonorrheal ophthalmia neonatorum, and Lancaster, Burnett and Gaus reported good results in conjunctivitis and also partly confirmed the earlier bactericidal laboratory results.

Mercurophen, or sodium oxymercury-orthonitrophenolate, according to Schamberg and his associates<sup>3</sup> is equal or superior to mercuric chlorid and other mercury compounds in germicidal activity, and maintains better its power in blood serum. It is a good disinfectant for instruments, which it does not

\* From the Ortho S. A. Sprague Memorial Institute and the Pathological Laboratory, University of Chicago.

1. Young, H. H.; White, E. C., and Schwartz, F. O.: A New Germicide for Use in the Genito-Urinary Tract: "Mercurochrome-220," *J. A. M. A.* **73**: 1483 (Nov. 15) 1919.

2. Clapp, C. A., and Martin, M. G.: Use of Mercurochrome-220 as a Germicide in Ophthalmia Neonatorum, *J. A. M. A.* **74**: 1274 (May 1) 1920.

3. Schamberg and others: *J. Infect. Dis.* **24**: 147, 1919.

tarnish, and for urine, pus, feces, sputum, catheters, rubber gloves and skin. It is less toxic for animals than other soluble mercurial compounds.

I have found that, while mercurochrome inhibits the growth of the tubercle bacillus completely in a dilution of 1:5,000, and partially at 1:10,000, mercurophen completely inhibits in dilutions of 1:50,000. Mercurochrome in a dilution of 1:100, but not in higher dilutions, killed tubercle bacilli in twenty-four hours so that they would not infect guinea-pigs. On the other hand, mercurophen, while it did not kill uniformly in less than twenty-four hours, did kill in dilutions of 1:10,000 in twenty-four hours; and the development of the disease in guinea-pigs inoculated with the exposed tubercle bacilli was generally delayed much longer than in the controls, even after shorter exposure to dilutions of 1:5,000 and 1:10,000.

In guinea-pigs inoculated with tuberculosis, treatment with either mercurochrome or mercurophen seemed to delay considerably the development of the disease; but marked generalized tuberculosis occurred in all the animals that lived long enough. Both drugs, if given by subcutaneous injection, caused infiltration, induration, necrosis and ulceration of the tissues around the point of injection. Apparently there was little absorption into the general circulation. Non-tuberculous animals treated in the same way showed no toxic effects, except the local effects described above.

While there seems to be but little beneficial action from either of these drugs in tuberculosis of guinea-pigs, it seems to me possible that mercurophen especially, because of its high bacteriostatic power, might be used with benefit in lupus and in ulcerating tuberculous conditions of the throat, larynx or bladder that are accessible for local treatment.

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# Weight Curves of Tuberculous Guinea-Pigs

Studies on the Biochemistry and Chemotherapy of  
Tuberculosis. XX

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LYDIA M. DEWITT

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# WEIGHT CURVES OF TUBERCULOUS GUINEA-PIGS

## STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. XX

LYDIA M. DEWITT

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In most of the experimental work on the chemotherapy of tuberculosis, the apparently favorable results of treatment are based on the following: Increased duration of life, favorable influence on weight, and diminished distribution and progress of the disease. If it were possible in this disease in guinea-pigs to attain the ideal of all chemotherapy—complete sterilization of the animal with a single dose, it would be unnecessary to judge our success in this way. But so far no drug has been found which will completely destroy all the tubercle bacilli in a guinea-pig without also destroying the animal.

Much stress has been laid on prolongation of life as an effect of treatment. In 1917, however, it was shown by Paul Lewis<sup>1</sup> that the duration of life in any series of tuberculous animals is too variable to be used as an indication of therapeutic activity, unless the number of animals used is very large and the individual variations are completely accounted for. He states that with guinea-pigs, he has yet to conduct an experiment in which the last animal to die did not live at least twice as long as the first to die, and often the difference is much greater than this. No one who has worked with tuberculous guinea-pigs or with other animals having experimental tuberculosis has failed to note similar uncertainties and differences in the length of life of the animals in any given experiment, even though the animals had all received the same dose of the same strain of tubercle bacilli, the same treatment throughout and had lived under the same conditions. It is not always possible to account for these variations. Tables 1 and 2 give some of the variations in the duration of the disease in comparison with variations in weights.

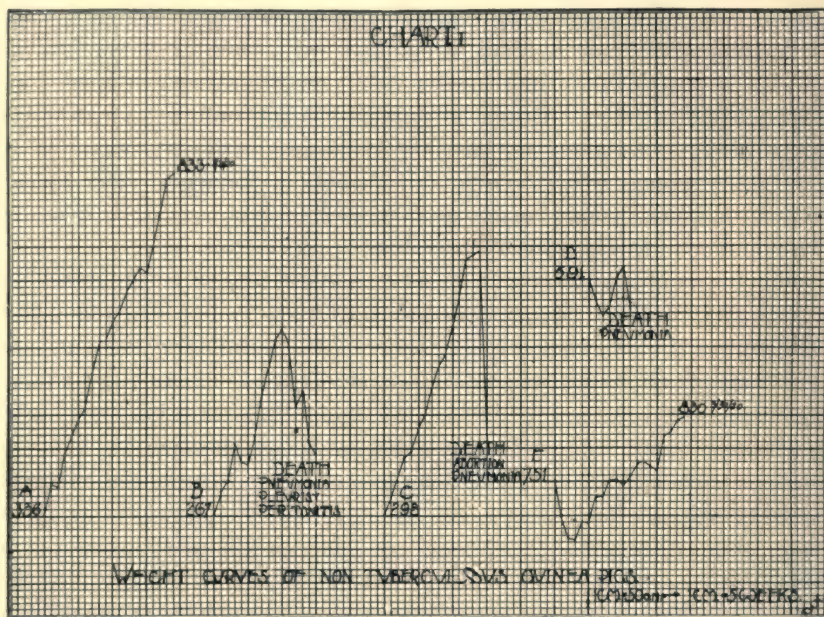
It is the purpose of this paper to endeavor to ascertain whether the weight curves of guinea-pigs inoculated with tuberculosis are any

<sup>1</sup> Fourteenth Report of the Henry Phipps Institute, 1918; *Am. J. Med. Sc.*, 1917, 153, p. 625.

more uniform than the duration of life and, if not, whether the variations can be more satisfactorily explained.

For a number of years, it has been my custom to have all animals weighed once a week as a guide in treatment. Some of the earlier weights I have discarded in this paper and have used chiefly those sets the weighing of which I have personally supervised.

In order to have a basis of comparison, I have, during the last 22 weeks, been weighing once a week a number of nontuberculous and of supposedly normal guinea-pigs, the initial weight of which was

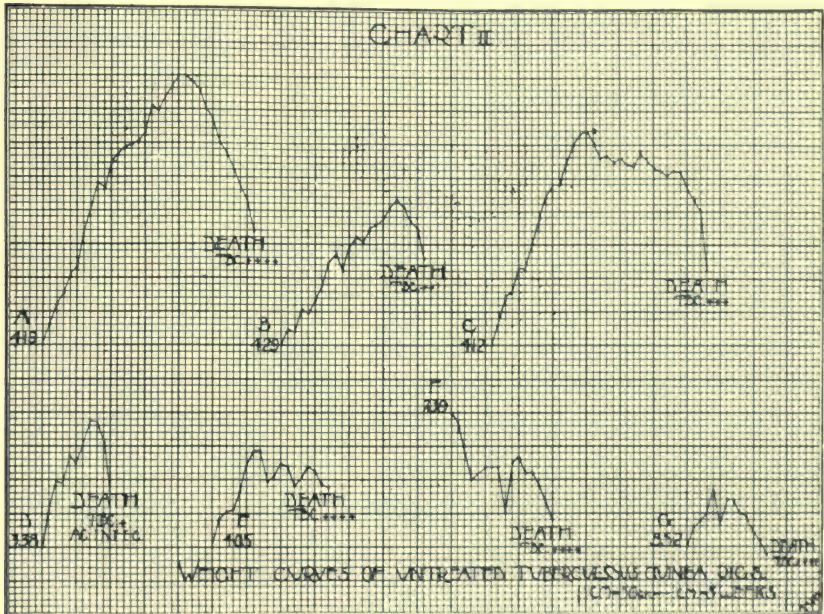


approximately the same as that of my inoculated animals. Chart 1 represents the type of weight curves of these animals with the cause of death in those cases in which death has occurred.

The curves in chart 1, as well as in the other charts, were made thus: The base line represents the weight of the animal at the time the experiment began—the initial weight line; this line is divided by vertical lines; one of the horizontal parallel spaces represents 10 gm. and one of the parallel vertical spaces represents one week. Nine supposedly normal guinea-pigs of different weights were used in this



experiment and were weighed once a week at the same time of the day, and, as far as possible, under the same conditions. Five of the animals died during the 22 weeks of this part of the experiment. Two died from infectious abortion with pneumonia, the weight curve (C, chart 1), showing a gradual rise as is typical of the normal growing animal and a sharp, sudden drop as seems typical of acute infections. Two, having a weight curve of the type of D, chart 1, died of an acute infection and one, having the curve B, chart 1, died from peritonitis and pneumonia. Of the 4 still living, 3 have a weight



curve typified by A chart 1, which seems to be the type of curve shown by normal, healthy, growing animals of medium initial weight. One of the heavy guinea-pigs, however, lost weight for about 3 weeks and then gradually gained and is still gaining (chart 1 E).

Chart 2 reproduces 7 of the most typical weight curves found in 37 tuberculous but untreated guinea-pigs. Table 1 itemizes the history of the individual animals used for chart 2, in which it may be noted that A, B, C and D are similar in contour, differing in height, in form of the summit, or in the width of the elevation according to

the rapidity of the ascent or descent and the length of life. An acute infection sharpens the summit and narrows the entire elevation. One of these four types is found 29 times among the 37 animals in table 1.

TABLE 1

TABLE OF WEIGHTS AND DURATION OF LIFE OF UNTREATED TUBERCULOUS GUINEA-PIGS

No. of Set	Duration of Disease, Days	Sex	Initial Weight, Gm.	Greatest Weight, Gm.	Terminal Weight, Gm.	Duration of Ascent, Weeks	Duration of Descent, Weeks	Type of Curve	Degree of Tuberculosis	Notes
1	129	F.	406	548	484	7	10	2 E	+++++	Acute infection
2	123	M.	386	569	408	10	6	2 A	+++++	
1	131	M.	375	509	357	11	7	2 A	+++++	
1	145	M.	437	589	465	10	4	2 C	+++++	
1	158	M.	429	640	508	17	4	2 B	+++++	
1	223	M.	419	815	589	20	11	2 A	+++++	
1	78	M.	338	534	432	8	2	2 D	+	
1	143	M.	336	605	412	8	5	2 C	+++++	
1	134	M.	481	635	394	6	10	2 C	++	
1	113	M.	345	605	531	12	8	2 D	++++	
1	98	M.	360	520	436	8	6	2 B	+++++	
1	246	M.	412	730	522	15	10	2 C	+++++	
1	158	M.	306	568	340	15	6	2 A	+++++	
1	167	M.	455	671	440	14	8	2 A	+++++	
1	130	M.	430	686	457	11	5	2 D	+++++	
1	116	M.	454	612	495	8	7	2 D	+++++	
1	97	F.	388	533	368	5	7	1 B	++++	
1	103	M.	410	497	422	5	6	2 G	+++++	
2	167	M.	352	599	432	15	9	2 A	+++++	Acute infection
2	169	M.	382	563	417	15	9	2 B	+++++	
2	164	M.	440	674	608	15	3	2 C	+++++	
2	171	M.	484	730	495	10	14	2 C	+++++	
2	113	M.	375	467	336	7	9	3 J	+++++	
2	101	M.	287	441	335	9	6	2 D	+++++	
3	74	F.	266	427	321	8	3	2 D	+++++	Acute infection Killed
3	74	M.	264	384	292	8	3	2 D	+++	
3	148	M.	215	401	384	12	9	2 E	++++	
3	112	M.	177	344	274	14	2	2 C	+++++	Acute infection
3	109	M.	259	391	313	10	5	2 A	+++++	
4	27	F.	590	680	660	2	1	2 D	++	
4	60	F.	240	290	205	4	2	3 B	+++++	
4	104	M.	295	425	255	10	3	3 C	+++++	
4	105	M.	580	685	610	7	3	2 E	+++++	
4	105	F.	730	730	500	0	15	2 F	+++++	
5	52	M.	352	438	341	4	6	2 G	++++	
5	76	M.	352	395	309	5	5	2 G	+++++	
5	69	M.	378	410	310	2	6	3 A	+++++	

Set 1 of table 1 consists of 18 guinea-pigs, varying in weight between 350 and 450 gm., which were inoculated at the same time with the same amount of the same strain of human tubercle bacilli. Six received the ordinary laboratory feeding of carrots, oats and hay. Twelve received lettuce and specially prepared graham crackers and hay. Hence the animals of set 1 differed only in their food. The weight curves of 16 of the 18 can be classed under the 4 types which I regard as typical for tuberculosis in guinea-pigs. One had the weight curve E, chart 2, which differs from C only in the fact that



death occurred very near the summit of the elevation and that the ascent was not so high. One had the curve G which differs from B in being lower and in having a double apex. Set 2, table 1, is composed of the 6 untreated controls of our cresol experiments. They were inoculated with the same strain of human tubercle bacilli as was set 1, but received four times as large a dose. Five of these had curves of one of the four main types, but one, which developed an acute peritonitis in addition to the tuberculosis, showed a curve similar to J, chart 3, which is formed much like the typical curve, but is lower and descends below the base line.

Set 3 consisted of 5 guinea-pigs inoculated with the washings from garnets used as controls in a bactericidal experiment. The garnets were covered with an unknown quantity of tubercle bacillus suspension, then exposed to salt solution, washed and shaken in salt solution. Hence the dose used in the inoculation was unknown. However, of the 5 animals whose curves were taken, 4 had the typical curves A, C and D, while one had curve E, in that it was killed near the apex of the curve. Set 4 was inoculated with an unusually large dose of a very virulent strain. As a result, the duration of the disease was relatively short and the curves less typical than in the other sets. Set 5 was also a bactericidal control. Only three curves were used, since most of the animals are still living. Curve G reproduces the curve in two of these. The sudden drop shown in this curve was due to the fact that we had had no carrots for 4 days, so that during this time they had lived on oats and hay. The same explanation applies to curve A, chart 3, which more nearly resembles the curve of the third animal. In studying table 1 and chart 2 in the effort to determine the causes of the typical curves and of any modifications from the types, we note that most of the animals were young adults, still growing but past some of the weaknesses which belong to the young. Extreme age, as indicated by 2F and 1E, tends to modify the curve considerably. The natural tendency of the weight curve in the normal growing animal is constantly upward, as shown in A, chart 1, and since the inoculation with a small or moderate dose of a moderately virulent strain of human tubercle bacilli does not materially influence health at first, the curve follows its natural upward tendency until something in the way of diet or secondary infection or an overpowering of the animal with the effects of the tubercle bacillus impairs the health and the curve begins to descend. Larger initial doses or a more



virulent strains of organisms tend to cause shorter ascending curves and thus a lower elevation. Secondary infections may cause sharp, sudden descent. Sex seems to have some effect on the shape of the curve, since of the 6 females in table 1, 4 had more or less atypical curves, while only 8 of the 30 males had curves varying in any way from the typical.

It is not always possible to explain variations. It is even difficult to explain the typical curve. The ascent is easy to explain since that is natural. But why the occasional sharp breaks in the ascent? Why the final slow or rapid descent? Why the sharp or rounded apex in some cases and the broad but jagged plateau in others. We have already seen how variations in the diet may cause decided drops in the weight and these or slight infections may explain the occasional jagged outline of the ascent. The final descent does not necessarily mean the generalization of the tuberculosis, since several animals that died or were killed at or near the apex of the curve showed all the internal organs involved in the tuberculous process. We must, therefore, assume that the descent is due to the overpowering of the animal by the toxic products of the bacteria. Whether the apex shall be broad or narrow probably depends on whether the resistance gives way suddenly or gradually—in other words, on the individual factor in the equation.

The question of the effect of treatment on the weight curve can be answered only so far as it concerns the 56 cases whose weight curves have been plotted. No generalization can be made from this number since it is self-evident that the effect of treatment on the weight curve as well as its effect on the condition of the animal, on the progress of the disease and on the length of life must vary with the treatment. Each drug used will probably affect the curve differently.

Chart 3 gives in graphic form some of the types of curves found in the 56 animals which had received six different modes of treatment. The curves were plotted as in charts 1 and 2. Table 2 gives in detail the individual animals of these various sets, with the treatment of each, the duration of life, the weights and the type of weight curve shown, the numeral referring to the chart and the letter to the curve. Many of the curves varied so little from those of the untreated animals that the type in chart 2 or 1 can be used in describing these treated animals. Chart 3 therefore reproduces in the main those types





TABLE 2

TABLE OF WEIGHTS AND DURATION OF LIFE OF TREATED TUBERCULOUS GUINEA-PIGS

Duration of Disease, Days	Sex	Initial Weight, Gm.	Greatest Weight, Gm.	Terminal Weight, Gm.	Duration of Ascent, Weeks	Duration of Descent, Weeks	Type of Curve	Degree of Tuberculosis	Treatment
113	F.	562	879	580	10	3	3 F	++++	Orthocresol
188	F.	460	562	314	13	12	3 G	++++	Orthocresol
113	M.	553	664	475	8	9	2 B	++++	Orthocresol
87	F.	427	613	401	9	4	2 D	++++	Orthocresol
142	F.	586	822	754	19	2	2 B	++++	Orthocresol
118	M.	324	480	387	8	9	2 A	++++	Orthocresol
98	M.	355	551	434	8	5	2 A	+++	Metacresol
74	F.	575	654	436	7	4	3 J	++++	Metacresol
87	M.	362	535	425	9	4	2 B	+++	Metacresol
107	M.	415	513	440	5	3	3 E	+++	Metacresol
80	F.	535	611	425	2	6	2 I	++++	Metacresol
73	M.	503	554	357	4	6	3 J	+++	Metacresol
170	M.	415	556	440	14	8	2 C	++++	Paracresol
120	M.	399	491	373	10	4	3 E	+++	Paracresol
134	M.	429	534	409	16	2	3 H	++++	Paracresol
162	M.	390	534	400	12	5	3 E	+++	Paracresol
134	M.	344	561	457	17	2	2 B	+++	Paracresol
58	M.	455	556	402	3	5	3 J	+	Thymol
80	M.	483	554	396	6	6	3 I	+++	Thymol
115	M.	396	547	419	10	6	2 C	+++	Thymol
136	M.	390	553	394	8	12	2 B	++++	Thymol
147	M.	357	486	337	9	11	2 A	+++	Thymol
	M.	413	...	...	...	...	1 A	.....	Thymol
89	M.	420	490	442	6	4	2 E	+++	Mercurophen
139	M.	368	650	617	16	3	2 C	++++	Mercurophen
71	M.	367	501	387	8	2	2 D	+	Mercurophen
107	M.	430	551	498	7	1	2 C	+	Mercurophen
97	M.	384	465	382	10	4	3 E	++++	Mercurophen
132	M.	428	549	397	12	6	3 E	++++	Mercurophen
120	F.	380	515	435	11	6	3 F	+++	HgCl <sub>2</sub> blue methylene
114	M.	510	665	505	12	5	2 B	++	HgCl <sub>2</sub> blue methylene
68	F.	565	610	390	1	6	3 B	+++	HgCl <sub>2</sub> blue methylene
87	F.	500	650	385	5	6	3 C	++++	HgCl <sub>2</sub> blue methylene
75	F.	575	615	425	3	7	3 B	+++	HgCl <sub>2</sub> blue methylene
68	F.	480	570	520	9	3	3 K	+++	HgCl <sub>2</sub> blue methylene
76	M.	480	515	335	5	5	3 B	+++	HgCl <sub>2</sub> blue methylene
56	F.	440	455	330	2	5	3 I	+++	HgCl <sub>2</sub> blue methylene
75	F.	585	710	510	4	4	3 G	+++	HgCl <sub>2</sub> blue methylene
62	M.	528	565	358	4	6	3 A	+	HgCl <sub>2</sub> blue methylene
73	M.	572	572	364	3	6	2 F	+	HgCl <sub>2</sub> blue methylene
142	M.	450	550	445	8	8	3 E	++++	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
129	M.	695	765	545	6	10	3 F	++++	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
156	F.	620	665	530	5	5	3 G	++	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
143	M.	645	695	585	6	9	3 D	+++	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
153	M.	690	725	650	7	6	3 A	+++	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
85	F.	520	540	410	2	8	3 B	++++	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
86	M.	685	710	555	3	4	3 B	—	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
110	F.	445	525	495	12	3	2 E	++++	Tuberculosis vaccine and HgCl <sub>2</sub> methylene blue
60	F.	575	575	325	0	10	2 F	++++	Iodine in starch
100	M.	675	685	560	4	7	3 J	++++	Iodine in starch
78	M.	560	620	510	4	5	3 J	++++	Iodine in starch
77	F.	625	625	470	3	6	2 F	++++	Iodine in starch
90	M.	415	535	335	7	6	2 E	++++	Iodine in starch
95	M.	470	530	395	5	8	2 G	++++	Iodine in starch
138	M.	409	469	334	9	9	3 E	++++	Iodine in milk
117	M.	341	462	321	8	8	3 E	++++	Iodine in milk

\* Acute infection



living, but their weight curves up to the present time do not conform to the types that we have regarded as typical for untreated tuberculous animals. Two sets of animals have been treated with iodine. In the first set, the iodine was given in powdered starch and in these the curves were all more or less atypical. Of the second set, which have been fed iodine in milk powder, only two have died, showing the low but typically shaped curve represented in E of chart 3. Five of the guinea-pigs of the second set are still living and so far their curves correspond with the normal curve A in chart 1 or with the beginning of A or C of chart 2.

In order to determine how much of the effect on the weight curve in the treated animals was due to the treatment and how much to the treatment combined with the tuberculous infection, 3 normal guinea-pigs have received the same treatment as each set of pigs given in table 2 with the exception of the iodine in powdered starch and the tubercle bacillus vaccine combined with the double salt of mercuric chlorid and methylene blue. Of the 21 normal guinea-pigs thus treated whose weight curves have been thus charted, 4 have died of acute infections and have the curve of most acute infections. The rest are living after 3 and 5 months' treatment and their weight curves correspond with A of chart 1. In other words, if uninfected, they run, in spite of the treatment, the typical weight curve of the normal animal; that is to say, while these drugs modify considerably the weight curves of tuberculous animals, they have not, during the months of the experiment, changed the weight curves of normal, uninfected guinea-pigs. In the 37 untreated tuberculous animals, the height of the elevation, i. e., the difference between the initial weight and the maximum weight averaged 166 gm. and, if sets 4 and 5 of table 1 be omitted because of the larger dosage and greater virulence of the infectious organisms, the average height of the elevation is 195 gm. In the 55 treated tuberculous guinea-pigs, on the other hand, the average height of the elevation was only 100 gm. If, however, we wish to compare the average elevations of the tuberculous animals under the different methods of treatment, we find the average weight elevation of the 22 animals treated with the cresols and thymol is 139 gm.; the 6 tuberculous animals treated with mercurophen have an average weight elevation of 135 gm. The tuberculous animals treated with the double salt of mercuric chlorid and methylene blue have an average weight elevation of only 75 gm., while that of the 8 animals

treated with the same double salt and a vaccine of killed tubercle bacilli is 53 gm. Fifty-four grams is the average weight elevation of the 8 iodine-treated pigs.

If we compare the treated animals with the untreated with reference to the descent of the weight curve below the line of the initial weight, we find that only 11 of the 37 untreated animals had curves descending below the base line, while 37 of the 55 treated animals had such long descending curves. The average distance of the terminal weight below the initial weight in the untreated animals was 56 gm., while only 1 animal had a curve descending more than 100 gm. below the base line. The treated animals, however, showed an average distance below the base line of 95 gm., while 18 of the 37 had curves descending more than 100 gm. below the base line.

#### CONCLUSIONS

Normal guinea-pigs of approximately the same age and weight and living under the same conditions run a uniform weight curve. This curve is easily modified by changes in diet, by acute infections and other variations in the conditions of life.

Normal male guinea-pigs of approximately the same age and weight inoculated with the same dose of the same strain of tubercle bacilli and living under the same conditions, run a fairly uniform and typical weight curve. This weight curve may therefore be used in testing the effect of various methods of treatment and is a more reliable standard than the duration of life.

Most chemotherapy, so far as tested, even though the drugs and doses used are so nontoxic as not to interfere materially with the duration of life or with the weight curves of normal, uninfected guinea-pigs, tends to alter materially the type of weight curve. This alteration consists in the main in a diminution in height of the ascending curve and an increase in length of the descending curve.

It may be inferred that the more closely the weight curves of tuberculous animals treated by any method adhere to the normal weight curve, the more benefit we may hope for from the treatment.

## PRIMARY SPONTANEOUS TUMORS OF THE OVARY IN MICE

### STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

#### FOURTEENTH COMMUNICATION

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Throughout the animal kingdom the solid ovarian tumors seem to be infrequent, as they also are, relatively, among human tumors. Cystic tumors are described occasionally, but apparently less frequently in the lower animals than in man. Even among dogs, with their high incidence of tumor growth, ovarian tumors are rare according to the evidence furnished by literature dealing with canine neoplasms.<sup>1</sup> In Sticker's (2) compilation of tumors in domestic animals, of 766 tumors in dogs but 3 were in the ovary. As to other animals of Sticker's series, in 509 cases of tumors in horses, 4 were in the ovary; of 110 in cattle, 6 were ovarian; there was 1 ovarian tumor among 23 tumors in cats, and none at all among sheep, goats, and swine. Kimura (3) has reported 142 cases of tumors in horses, among which were no ovarian tumors, although there were 49 in the testicle. Other evidence supports the figures of Sticker in indicating that cows have ovarian tumors more often than other species. Trotter (4) reported 305 bovine tumors observed in the Glasgow slaughter house, of which 5 were in the ovary (4 carcinomas and

<sup>1</sup> Goodpasture (1) notes the occurrence of small hyperplastic areas in the senile ovaries of old dogs, but even these do not seem to be of very frequent occurrence in proportion to the high incidence of proliferative changes noted by him in other tissues.



1 sarcoma). These were all large lobulated solid tumors, and no metastases were observed in any. Leo Loeb (5) has described a tumor arising in the ovary of a six-months-old calf, composed chiefly of cells resembling luteum tissue.

Among the numerous instances of tumors in wild rats reported by McCoy (6), Woolley and Wherry (7), and Beatti (8) there is no case of ovarian growth; nor have we found reports of any cases occurring in domesticated rats.<sup>2</sup>

Wolff (9) mentions 2 cases of ovarian tumors in cats: One a primary carcinoma in the ovary of a thirteen-year old cat with metastasis in the liver, reported by Kitt; the other a sarcoma of the ovary and pelvis reported by Stroud.

Wild animals are also unlikely to have ovarian tumors. Fox (10), in his extensive studies of tumors in wild animals, has described no cases whatever of ovarian tumors among the mammalia.

Only in birds do ovarian tumors seem to be relatively frequent. Bürger (11) found, among 852 fowls autopsied at the Leipzig veterinary institute, 12 tumors, of which 7 were in the ovary, 4 being sarcomas and 3 carcinomas; 2 of the sarcomas and 2 of the carcinomas had produced metastases. In their review of the literature on tumors in birds, Joest and Ernesti (12) found 112 cases reported and added 50 more. Of these 162 cases, 21 were primary carcinoma of the ovary, commonly with extensive peritoneal and visceral metastasis. There was also one case of ovarian sarcoma.

An ovarian tumor has been described in a wild turkey (*Meleagris gallapavo*) by Fox (10), as follows: The growth was "about the size of 3 English walnuts placed in triangular position." It consisted of 3 subdivisions, covered with papillomatous prominences like a hydatid mole. Microscopically it was a papillary cystadenoma.

<sup>2</sup> While this article was in press there appeared under the title "Carcino-sarcoma de rata blanca," an article by Dr. A. H. Roffo (Revista del Instituto Bacteriologico, Buenos Aires, 1919, ii, 283), reporting the finding of a large tumor in the ovary of a white rat. This seems, from the illustration and description, to have been a papillary cystadenoma with areas of more compact cell growth, some of which are interpreted as carcinoma and some as sarcoma.



Cold-blooded animals also have furnished occasional cases of ovarian tumor. Bland-Sutton (13) has reported a case of tumor of the ovaries of a python, with growths also in the lungs, liver, and peritoneum; he believed the ovarian growth to have been primary but without conclusive evidence. Plehn (14) has described tumors arising in the ovary of an old grass frog (*Rana esculenta*) through growth of primitive ova cells without differentiation. There were numerous nodules, from millet seed to cherry size, apparently benign in character although resembling a malignant tumor in histological structure. A cystic tumor of the ovary has been described in a fish, the ling (*Molva molva*) by Johnstone (15).

In mice, also, ovarian tumors are not common, but we have found mention of 8 cases in the literature. The first 2 cases were described by Jobling (16). One was bilateral, each ovary being eight times the normal size. Both ovaries showed the same structure, which is described as follows:

A great increase in epithelial cells, which formed solid masses, somewhat compressed and elongated into spindle cells and cysts of different sizes, usually small and more or less occupied by the papillary outgrowths from their walls. These outgrowths developed from narrow or somewhat thickened pedicles and spread out into a fanlike structure. . . . The more solid portions were at one time cystic but the cysts became occluded by the ingrowth of papillae. Acini possessing a distinctly granular [sic., glandular?] form and arrangement also occurred. Mitosis was rare, direct division more frequent. Hemorrhage had taken place into some of the cysts. Mallory stain showed a delicate connective-tissue basement membrane surrounding the small cysts and the more solid areas, the latter being filled with epithelial cells of a granular quality resembling somewhat the lutein cells.

In the other case the growth was unilateral, there being numerous large cysts, separated by smaller ones and by the tubules of the ovary, lined by high columnar epithelium without cilia. Between the cysts the tissue was composed largely of smooth muscle fibers, resembling in places a leiomyoma.

Tyzzer (17) has reported 4 cases. 1. This tumor was bilateral, the ovaries being replaced by an irregularly glandular epithelial growth, in places with flattened epithelial cells intimately mingled with the connective tissue; in others the glands contained thick papillary processes of epithelium without central connective tissue core protruding into the lumen, the epithelium being in places high columnar, in others merely a fused mass. There were no mitotic figures, but the presence of masses of epithelium in the lymphatics suggested malignancy. 2. In a mouse with a renal tumor resembling a hypernephroma, one ovary was replaced by an irregular gland-like structure similar to the above. 3. A mouse of a series bred for heredity studies, which died from a lung tumor, had the right ovary replaced by a mass the size of a large pea, composed of irregular epithelium having in places a partially glandular structure, without tendency to form spaces. 4. A mouse from the same family as (3), had both ovaries replaced by masses of translucent whitish tissue, 5 by 3 by 3 mm. This also had the structure of a papillary adenoma. Tyzzer notes that these growths differ from the ordinary types of ovarian tumors seen in human beings, in that the epithelium is more or less glandular with but little tendency to form cysts. The epithelium resembles the peritoneal mesothelium found at the attachment of the ovary.

Haaland (18) found 2 ovarian tumors among 353 primary tumors observed in 288 mice, of which 325 were mammary gland growths. 1. A mouse that had a sarcoma arising in a scar also had a tumor the size of a hazel nut in the left ovary. The ovarian tumor was composed of large alveoli with a peripheral layer of low columnar epithelial cells, the lumen filled with round cells and sometimes exhibiting spaces filled with serous fluid. This tissue was transplanted into 120 normal mice, but no growths resulted. 2. A mouse that had been operated for mammary carcinoma with recurrence. A tumor "as large as a pea" replaced the right ovary, and in structure was a papillary adenocarcinoma, probably primary.

Of these 8 recorded cases of ovarian tumors in mice there were 3 cases of bilateral tumors, although in no case was there metas-

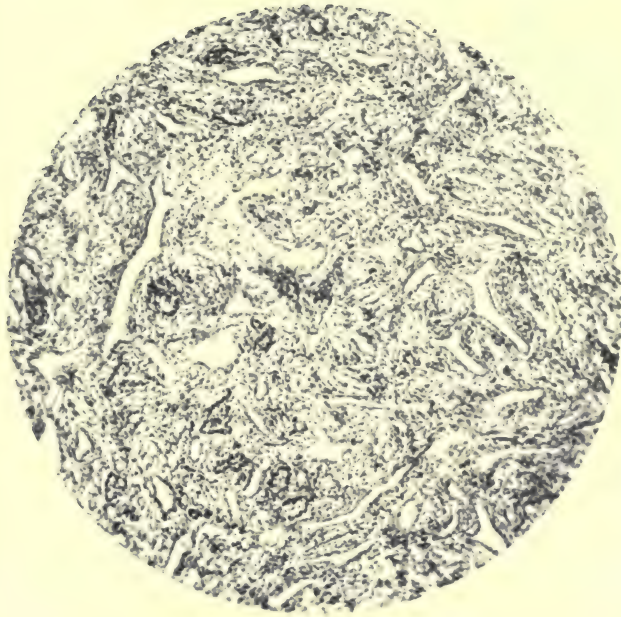


FIG. 1. SOLID TUBULAR ADENOMA

This is the most usual type of benign tumor of the ovary in mice. Much of what seems to be stroma between the tubules is really composed of compressed epithelial cells. Mouse 12760.  $\times 110$ .

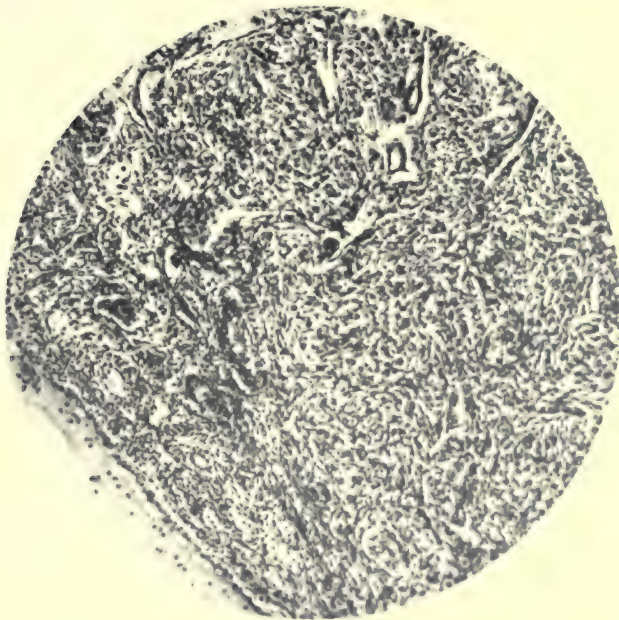


FIG. 2. SOLID TUBULAR ADENOMA

Showing a larger proportion of compressed epithelial masses.  $\times 110$



tasis or other positive evidence of malignancy. Four of the eight mice had tumors elsewhere than in the ovaries. All but one of the tumors were of similar structure, consisting of atypical glandular or alveolar formations with a tendency to epithelial proliferation into the lumen to form solid plugs or papillae—apparently best described as solid, atypical papillary adenoma.

Among 22,000 mice of the Slye stock that have come to autopsy have been found 46 with solid tumors that seemed to be primary in the ovary. This may be compared with 28 testicle tumors in 19,000 autopsies, 160 lung tumors in 6,000 autopsies, 87 sarcomas in 12,000 autopsies, and 4 cancers of the stomach in 16,500 autopsies.<sup>3</sup> These figures indicate that, as with other animals, solid ovarian tumors are not common in mice. There have been observed simple cystic conditions in the ovaries of but a small number of mice, and ovarian cysts are apparently rather infrequent. The strikingly large cysts seen in women have never been observed. In only a few of the

<sup>3</sup> We would again emphasize the character of the material from which these tumors have been obtained, and the conditions under which the growths have developed. The 22,000 mice are all the descendants of a limited and carefully selected stock, bred together according to definite plans designed to give evidence as to the influence of heredity upon the incidence of spontaneous tumors in mice, and, hence, including strains of highly cancerous ancestry and strains with ancestry free from cancer. They represent strains in which cancer is very common, strains in which it never occurs, and strains of intermediate character. The influence of heredity on the incidence of ovarian tumors will be considered elsewhere, and we mention these facts here to indicate the character of the material in this respect. It must also be emphasized that none of these mice has been subjected to any artificial influences that might modify its life. In no case is a spontaneous tumor used for inoculation, or operated upon, and no mouse born in this laboratory is ever used for any experimental work whatever. From the moment of its birth every effort is directed to the one object of permitting each mouse to reach a maximum age. Long experience and great care have made it possible to limit to a large extent the epidemic infections that constantly threaten such large colonies of mice under even the best of conditions. Of especial importance is the fact that every mouse that dies is submitted to a careful post-mortem examination, no matter whether it dies in infancy, from an accident, or from any other obvious cause; and every suspicious area is submitted to microscopic examination by three people or more. Were it not that every dead mouse is thus thoroughly investigated, and that the average age at death is, for a mouse community, very high, we should not have nearly so much material to describe.

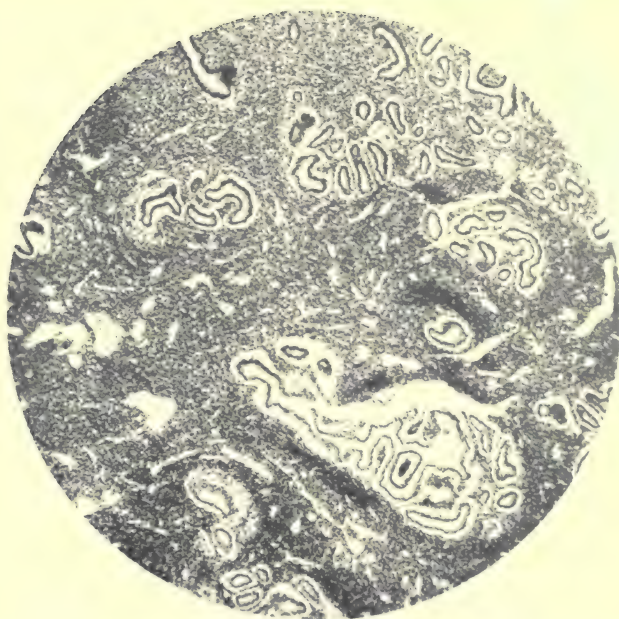


FIG. 3. SOLID TUBULAR ADENOMA

In this growth there is an unusually large proportion of ovarian stroma type.  
 $\times 76$ .

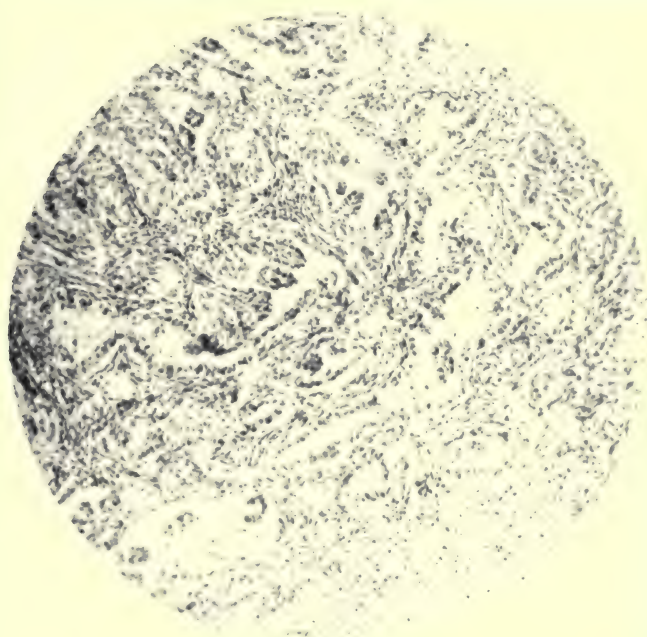


FIG. 4. PAPILLARY PORTION OF A GROWTH THAT ELSEWHERE IS A SOLID TUBULAR ADENOMA, AS IN 1 AND 2

Small areas thus disclosing an existing tendency to papillary structure are not infrequently found in the ordinary solid ovarian tumors of mice. Mouse 2205.  
 $\times 110$ .

ovarian cysts that we have examined have small cystic papillomatous areas been found. Therefore, mouse ovarian tumors present a marked preponderance of solid adenomatous growth, as compared with the proportion of cystic ovarian enlargements in women. We have eliminated from our consideration the simple cysts, as probably not examples of neoplastic growth, with the exception of those cysts that result from secretion by a cyst-adenoma.

Most of our tumors seem to be benign in character, although we have found a few examples of undoubted malignant tumors primary in the ovary. In all, 38 mice have exhibited tumors that may be classified as solid benign ovarian tumors, and there was one typical papillomatous cystoma. As 19 of these had bilateral tumors there are 58 ovarian tumors occurring in a stock of mice that have yielded over three thousand primary spontaneous tumors of other tissues, chiefly the mammary gland.

#### BENIGN OVARIAN TUMORS

In structure these tumors vary considerably, although most of them correspond closely to the descriptions given by Jobling, Tyzzer, and Haaland, and may be most appropriately designated as solid alveolar adenoma. Tendency to cyst formation is exceptional, in contrast to the adenomas of the human ovary, and the same is true of papillary types of growth, which are rarely exhibited. In general these solid adenomas of the mouse ovary exhibit a growth apparently under great pressure, with crowding of the cells so that it is usually difficult to differentiate readily between stroma cells and flattened epithelial cells (see figs. 1 and 2). Probably this crowding of the growth accounts for the absence of papillary tendency, for often a small area may be found where there is less pressure or where part of the tissue has been destroyed by hemorrhage or necrosis, in which a distinct tendency to papillary structure is seen (see fig. 4). We have only one case (12111) in which the structure corresponds at all closely with the human papillary cystoma (fig. 7).



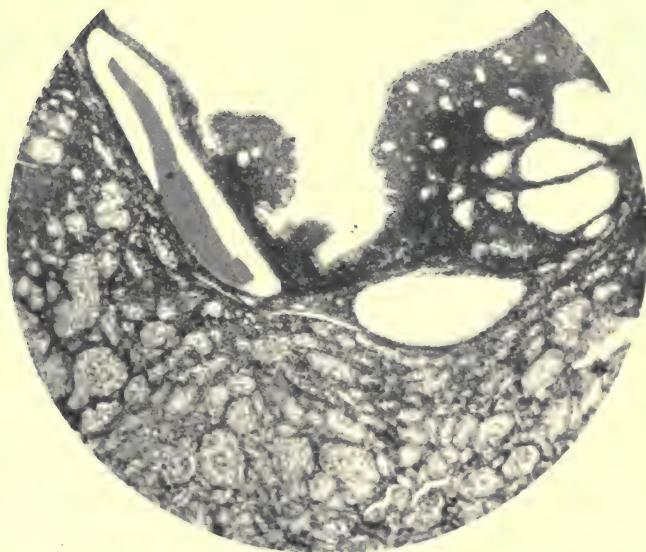


FIG. 5. OVARIAN TUMOR EXHIBITING CYSTADENOMA TYPE OF GROWTH IN ONE PORTION, WHILE ANOTHER PART SHOWS A SOLID ALVEOLAR CHARACTER RESEMBLING PRIMITIVE FOLLICLES  
Mouse 6991.  $\times 50$

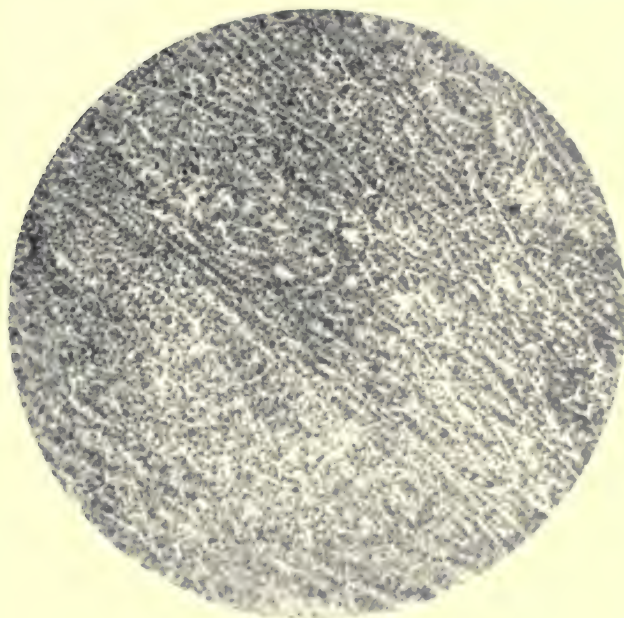


FIG. 6. SOLID TUBULAR ADENOMA

Consists throughout of a solid compressed mass of epithelial tubules, with a minimum of stroma. Mouse 20207  $\times 410$

Except for the few malignant growths in this series, nearly all the tumors seem to be fundamentally similar growths, yet differing considerably from one another according to the degree of differentiation permitted by the pressure under which the cells are growing. The usual character may be described as follows: The affected ovary is as a rule, uniformly enlarged, commonly to a diameter of 3 to 10 mm., white, firm, and often with a lobulated surface. In half of the cases the growth is bilateral, (19 of 38), usually one of the ovaries being considerably larger than the other.<sup>4</sup> The capsule is distinct but there may be adhesions to the adjacent tissues. If cysts are present they usually contain a clear watery fluid, but may have a blood-stained content. In most cases the benign ovarian adenomas have caused no apparent trouble; usually they are autopsy findings in mice dying from some unrelated condition. Sometimes fatal hemorrhage has occurred from ovarian tumors. In passing, it may be mentioned that large hematomas often form in the ovaries of mice without neoplasms, the blood usually forming strikingly laminated clots.

Microscopically the enlargement is usually caused by a compact growth of cells of two types, low cuboidal epithelial cells and spindle-shaped cells resembling those of normal ovarian stroma, but which often are definitely, in part at least, composed of compressed epithelial cells (see figs. 1 and 2). The cuboidal cells may form tubules, or solid plugs resembling the "Pflüger's tubes," or solid alveolar masses which may resemble primitive follicles, (see fig. 5), or occasionally alveoli into which papillary or fan-shaped outgrowths of the lining of the epithelium are crowded.<sup>5</sup> Most of the tumors show more or less of each of these types of growth, one type generally predominating. Sometimes one part of a section is composed solely of one type and another part of another type. Sometimes these various structures are

<sup>4</sup> This corresponds well with the figures given for human solid ovarian tumors by Massabuau and Etienne (19), who found that of 250 such tumors 43 per cent were bilateral. Of the eight cases of ovarian tumors in mice reported by Jobling, Tyzzer, and Haaland, three were bilateral.

<sup>5</sup> Such a type of growth is shown by Tyzzer (17) in his figure 24.

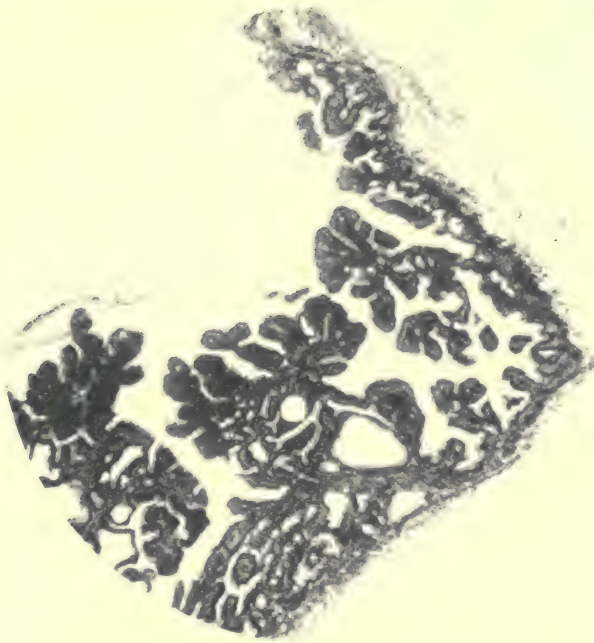


FIG. 7. PAPILLARY CYSTADENOMA

The only specimen in our series typically reproducing this type common in the human ovary. Mouse 12111.  $\times 45$ .

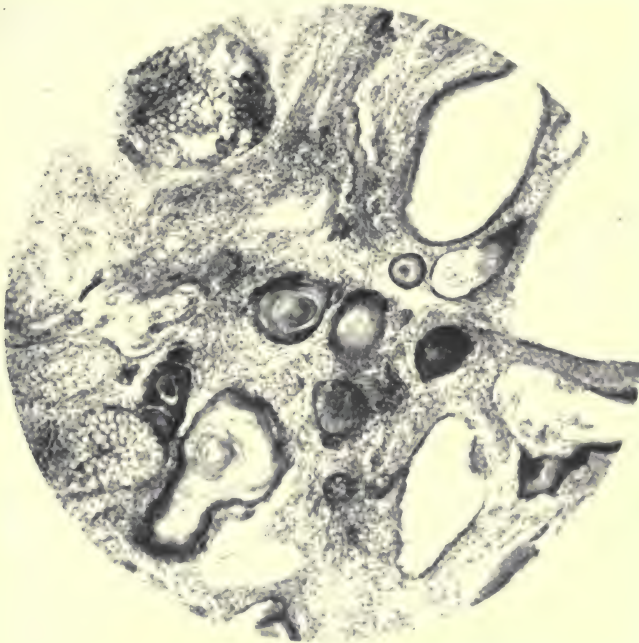


FIG. 8. SOLID TERATOMA

Shows in this field islands of cartilage, epithelial plugs with hornified epithelium, cysts lined with squamous and with columnar epithelium, and varying types of stroma elements. Mouse 9278.  $\times 60$ .



limited by a distinct basement membrane of true stroma cells, but often there seems to be a false stroma of crowded, spindle-shaped epithelial cells without sharp demarcation from the true stroma cells that may be present. Usually the total amount of stroma is small, most of the growth being composed of epithelial cells, and this stroma is of the cellular ovarian type and not ordinary fibrous tissue. Not infrequently, however, the stroma elements form the bulk of the growth (fig. 3). Generally the capsule is formed by compressed ovarian tissue, in which ova or follicles are rarely seen. Blood vessels are usually scanty; but, nevertheless, necrosis or other retrogressive change is seldom found. Mitosis is very rarely observed, nor are forms suggestive of amitotic division common. The epithelium resembles the germinal epithelium and occasionally the surface of the tumor is covered with this cuboidal epithelium which dips down at intervals to divide the growth deeply into lobules.

As variations from the structure described above we have in a few instances the formation of several small cysts with lining of flattened epithelium. In a few also the structure suggests a papilloma with all spaces obliterated by pressure or from lack of secretion by the surface epithelium. If the growth is of tubular character the pressure usually causes the lumens to resemble narrow slits (figs. 2 and 6). In some instances the alveolar structure is filled with flattened cells consisting chiefly of deeply staining nuclei, producing a growth resembling the adenomatous growths often found in the human vermiform appendix. In only one instance have we a true fibroadenoma (12922) in which a large part of the tumor is composed of definite fibrous stroma with abundance of collagenous material, rather than the cellular ovarian type of stroma. We have not identified any of our tumors as of lutein cell structure, nor have we observed fibromas or myofibromas in the ovary.

#### MALIGNANT OVARIAN TUMORS

We have found the following tumors that seem to be unquestionably primary malignant tumors arising in the ovary.

6487. The left ovary was 10 mm. in diameter, nearly spherical, partly white and fleshy, partly cystic. There were no

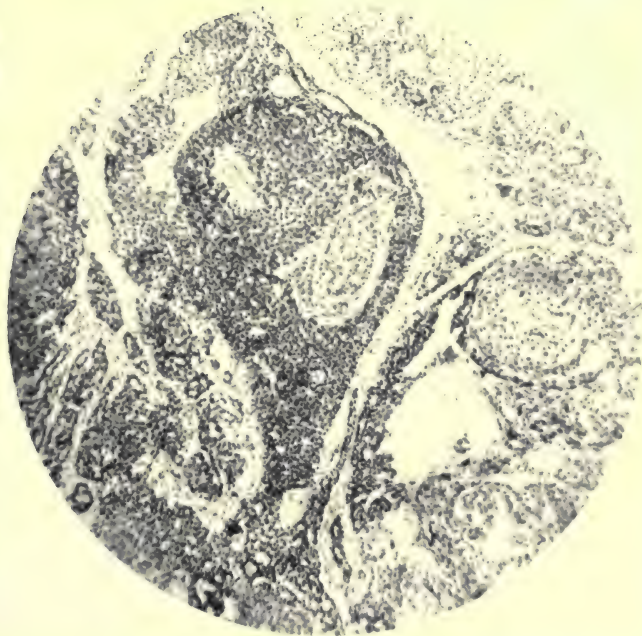


FIG. 9. MALIGNANT EPITHELIAL TUMOR OF OVARY  
Showing both tubular and alveolar types of growth. Mouse 6487.  $\times 100$

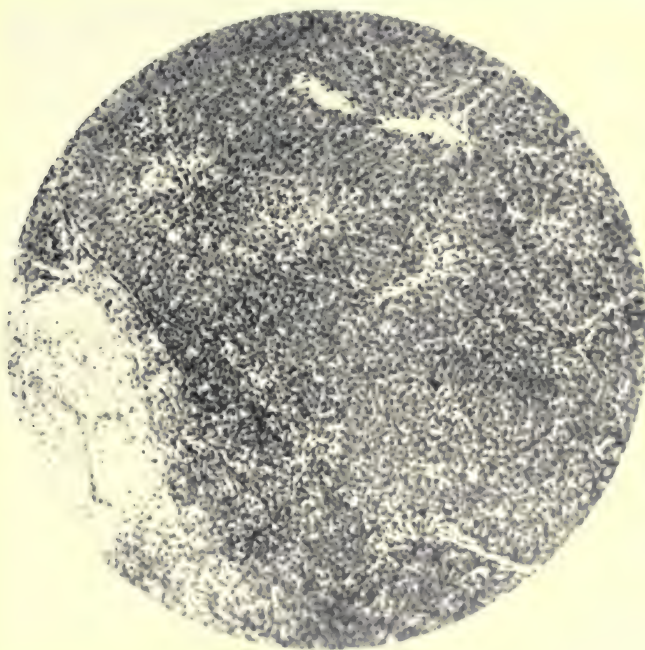


FIG. 10. MALIGNANT EPITHELIAL TUMOR OF OVARY  
Showing solid type of growth of large cells without any well defined arrangement. Similar structure is also shown by tumors of testicle and adrenal. Mouse 12552.  $\times 110$ .



adhesions and no evidence of metastasis. The mammary gland exhibited a large hemorrhagic carcinoma and also a minute early carcinomatous nodule, both of these growths being typically and unquestionably primary carcinomas of the mammary gland; structurally they were entirely distinct from the ovarian growth. About one half of the latter was composed of a cavity filled with a protein-rich fluid, and with walls formed by an irregular border of tumor tissue. Apparently this cyst was formed by degeneration of tumor tissue, as it had no proper cyst wall, although on the side towards the tumor there are some papillary outgrowths into the cavity. The solid part of the tumor consists of epithelial cells arranged mostly in large irregular alveoli, often exhibiting central necrosis, and occasionally atypical tubules are seen (fig. 9). The stroma is scanty and of ordinary connective tissue type for the most part, although these are areas with abundant elongated cells. The cells are large with a clear cytoplasm, well defined borders, and oval nuclei which are partly solid and partly slightly vesicular. Occasional mitotic figures are seen. Although there are no metastases, the histological structure of this tumor is such that it must be considered to be malignant, an alveolar carcinoma of the ovary.

12552. The left ovary measured 25 by 18 by 18 mm., nodular, with many distended blood-vessels coursing over it. It was not adherent to adjacent tissue and no metastatic growths were found. In the mammary gland were two hemorrhagic carcinomas, entirely different in structure from the ovarian tumor. Microscopically the ovarian tumor is composed of large irregular alveoli filled with large cells with a moderate amount of cytoplasm and a large pale nucleus, with very little stroma (fig. 10). Several mitotic figures were found. The original ovarian capsule surrounds the growth, but no recognizable ovarian tissue remains. Areas of necrosis and hemorrhage are numerous. This tumor closely resembles 6487 but exhibits a greater degree of malignancy.

6801. The left ovary was converted into a hemorrhagic nodular mass, 18 by 15 by 10 mm. The right ovary was normal.



A mass of tumor tissue lay anterior to the left kidney, about the size of the kidney itself. There was a large carcinoma of the mammary gland arising in the left flank, and producing metastases in the lung, as shown by microscopic examination.

The large ovarian tumor consists of about equal parts of extravasations of blood or large dilated blood channels and of tumor tissue. Nothing of the original tissue remains, except that in the capsule appear a few cells suggestive of ovarian stroma elements. The tumor has no well defined structure, consisting of large solid masses of cells which occasionally are formed into trabeculae by blood channels, but otherwise show no tendency toward grouping of any kind. The cells are characterized by a considerable amount of cytoplasm which is usually homogeneous and often with well defined cell borders. The nuclei are round, sometimes solid and sometimes vesicular. Where the cells are largest they resemble slightly liver cell types. There is practically no stroma besides the blood vessels, which are scanty except for the large channels in the tumor without vessel walls. The retroperitoneal mass presents quite the same structure. Mitotic figures are not seen. A few multinucleated cells are found. The general character of the growth resembles that of other malignant tumors found in the adrenal and testicle.

14099. The left ovary is about three times the normal size, and of a red color. The uterus is distended with a milky fluid. In the upper lobe of the right lung is a firm nodule, 2 mm. in diameter, which proved to be a simple papillary adenoma, apparently benign but with a projection growing into a blood-vessel. There were no changes of significance in the other organs. Microscopically the ovary is found to be almost entirely replaced by a mass of large cells with no particular arrangement, which invade the remaining recognizable ovarian tissue, and in which appear epithelial structures that resemble overgrown Pflüger's tubules. The nuclei are deeply stained and irregular in size. In the perirenal tissue is a small growth of similar character.

It is interesting to observe that the malignant types of tumors arising from the ovary, testicle, and adrenal that we have studied exhibit such a similar histological picture, and one quite dis-

similar from tumors arising in other tissues. There can be little doubt that they all represent reversions to the primitive embryonal tissues of the urogenital anlage, and are probably best designated by Adami's term, mesothelioma. This similarity of structure makes it difficult to determine the origin of a tumor involving both the ovary and the adrenal, as in the following case.

12307. The abdominal cavity shows several nodules whose exact origin is difficult to determine as the mate has partly devoured the body. The right ovary is, however, easily distinguished. It measures 18 by 12 by 12 mm. What seems to be the left ovary is 10 by 8 by 6 mm. There are 8 other similar nodules in the abdominal cavity, one being in the position of the left adrenal, measuring 10 by 8 by 8 mm. The other nodules are apparently in the mesentery. One lobe of the liver is converted into a tumor nodule 14 by 10 by 18 mm., irregular and lumpy in outline, pink in color.

The tumor tissue shows everywhere the same structure, consisting of irregular alveoli composed of large cells with abundant cytoplasm with well defined borders and deeply staining nuclei. Mitotic figures are numerous. The character is that usual to mesothelial growths. The ovary cannot be positively identified, but one mass exhibited in the capsule structure suggests compressed ovarian tissue with degenerated ovum. In all respects this tumor is identical with the malignant ovarian tumors just described.

It seems probable that this tumor arose in the ovary which exhibited the largest growth, but it is not possible to exclude the adrenal as the primary site.

Still more difficult to locate is the primary growth in the following case.

12876. The left kidney contains a mass of pink, fleshy tissue, 18 by 14 by 14 mm. The right kidney, which is slightly enlarged, contains no tumor mass. The right ovary consists of a pinkish tissue resembling that in the kidney, and measures 12 by 8 by 8 mm. In the mesentery is a similar, slightly paler mass, 16 by 8 by 8 mm. The retroperitoneal and subcutaneous glands are not enlarged and no nodules are found in the lungs.

Microscopically the tumor tissue is alike in all three places, consisting of a diffuse infiltrating growth of large round cells, which also invade the connective tissues about the kidney and ovary. It does not at all resemble the typical ovarian tumors, being apparently a round-cell sarcoma. We have no way of telling which of the three tumors was primary. The next case presents similar difficulties.

26. This mouse had a tumor mass about 8 by 10 mm. in the upper portion of the liver, with other smaller nodules near it. A similar small nodule was found in the right kidney. The right ovary was enlarged to two-thirds the size of a kidney, and was solid. Microscopically all these growths are composed of round cells, apparently a round-cell sarcoma. It is impossible to say which growth was primary.

We have seen few instances of secondary tumors occurring in the ovary. The Krukenberg tumor is not found because mice do not have gastric or other abdominal carcinomas, except most rarely (20).

In leukemia and pseudoleukemia, infiltration of the ovary with lymphoid elements is common, and often very striking. In our collection there has been no case observed of secondary carcinoma of the ovary, which is not surprising in view of the relatively slight tendency of mouse carcinomas to produce metastasis elsewhere than in the lungs. The following examples of secondary sarcoma have been noted:

12058. A bilateral sarcoma of the uterus, round-cell in type, with metastasis in the right kidney. The left ovary was 10 mm. in diameter and showed complete replacement by sarcoma tissue. As near as we can determine the growth in this case arose in the uterus, and invaded the ovary by infiltration.

19061. A spindle-cell sarcoma growth infiltrated the retroperitoneal tissues extensively, including the pancreas, also with metastasis in the liver. Apparently primary in either the retroperitoneal tissues or in the mesentery. The uterus and both ovaries were infiltrated by the same tissue.

To summarize, we have found four malignant growths that seem to be certainly primary in the ovary. Each of these



exhibited the structure common to malignant tumors arising in the sex glands and adrenal. One exhibited retroperitoneal metastasis near the kidney. Another tumor of similar histological type also involved the adrenal, and produced numerous metastases; in this case it was not possible to determine whether the growth was primary in the ovaries or in the adrenal.

There were two round cell sarcomas involving the ovary as well as other organs, which might have been primary in the ovary, but the evidence did not permit of deciding this. There were two other cases in which the sarcomatous invasion of the ovary seemed to be unquestionably secondary. No secondary carcinomas were observed in the ovary.

#### TERATOMA OF THE OVARY

From the accounts of animal tumors in the literature it would seem that teratomas are extremely infrequent in the lower mammals. In reviewing the literature on the occurrence of tumors of the ovary and testicle, in the lower animals the chief sites of teratomatous growths, we have found mention of but one case. That was described by Winokuroff (21) as a teratoma of the testicle in a chicken, the growth exhibiting cartilage, bone, striated muscle, squamous epithelium, and cysts. Our own experience supports the view that the lower animals rarely exhibit teratomas, for in the 22,000 mice here considered containing over three thousand spontaneous primary tumors, we have observed but one teratoma. This arose in the ovary, and is a typical example of solid teratoma as shown by the following description:

9278. Death resulted from pulmonary infection. There were no other changes of importance except in the left ovary, which measured 20 by 18 mm., and yielded an exudate from the cut surface. Microscopically the growth has a delicate but distinct capsule in one portion of which there still remains a trace of the original ovarian tissue with one follicle containing an ovum. Except for this the entire section shows a mass of tissues of all sorts thrown together in an entirely disordered

manner (fig. 8). There are numerous small cavities, some of which are lined with squamous epithelium containing chiefly desquamated cells and some polynuclear leukocytes. Occasional solid plugs of squamous epithelium also occur. In places these are branching and occasionally a basal-cell type of growth is seen. No true hair follicles are recognized although occasionally the epithelial downgrowth suggests these structures. No sebaceous glands are found. Most of the squamous-cell structures are grouped together in localized areas. There are also spaces lined with columnar or flattened epithelium, sometimes with a content resembling a diluted mucin. Spaces are found in which part of the lumen is lined with stratified epithelium and part by cuboidal or columnar epithelium. Sometimes these tubules have a well defined coat of non-striated muscle fiber. Small islands of cartilage are abundant, usually having no definite relation to other structures. No bone is seen. Some of the cavities contain old extravasations of blood in varying stages of disintegration. In one place there is a mass of heavy brown pigment near which are collections of small round cells; the whole appearance suggests that possibly this area represents undeveloped retinal tissue. The stroma in general consists of a loose fibrous tissue with abundant cells. There are also many cells with much more cytoplasm than ordinary connective tissue cells, this cytoplasm having a slight basophilic tendency. No striated muscle is found or definite organ tissues but there are many accumulations of cells which are not stroma cells and which presumably are undifferentiated cells of special tissues.

Since this analysis of 22,000 autopsies was made, another instance of teratoma of the ovary has been observed, so that we now have found two teratomas among 25,000 autopsied mice. The chief features of this second case are as follows:

24172. Mouse, eleven months old, died apparently from acute pulmonic infection following delivery, but when found post-mortem changes were too far advanced to be certain of the diagnosis. The puerperal uterus showed no gross evidence of infection. There were no tumors except that involving the right ovary, which measures 30 x 15 x 15 mm., and is nodular,



encapsulated, but not adherent. The mass is soft in consistency and heterogeneous in appearance. There is no exudate or cyst formation on the cut surface.

Microscopically the growth is found to be composed of many different sorts of tissue elements, but unfortunately a large part has undergone so much necrosis and post-mortem change that the structures cannot be well studied. In the portions that do stain the elements are extremely varied and without any particular relation to one another. The greater part of the tissue elements cannot be identified as to their origin or character, being merely masses of cells with small nuclei and considerable cytoplasm. Conspicuous are the plugs of squamous epithelium, with masses of hornified material in the center, but recognizable skin, hair follicles, or sebaceous glands are not found. Tubules lined with columnar epithelium are also occasionally found. There is unusually little tendency to form epithelial-lined cavities or cysts. Numerous areas of mucoid degeneration are present, but goblet cells are not present in these areas, nor can the cellular origin of the mucin be determined. There are a few small islands of bone tissue, but only a few minute groups of cartilage cells. Nonstriated muscle fibers are abundant, usually in distinct bands, but striated muscle fibers are not found. A very little fatty areolar tissue is present. Except for fibrous tissue and blood vessels these are all the tissue elements that can be identified, although some areas resemble liver cells, and others simulate developing nervous tissues. Nothing suggestive of malignancy is found. The diagnosis is clearly solid teratoma of the ovary.

#### COEXISTENCE OF OVARIAN TUMORS WITH OTHER TUMORS

As emphasized in previous papers of this series, mice with tumors in one tissue exhibit tumors in other organs with a frequency greater than would correspond to the average incidence of tumors. It may be recalled that of the 8 cases of ovarian tumors recorded in the literature, in 4 there were tumors elsewhere in the body.



Among our 40 mice with benign ovarian tumors, 22 had tumors elsewhere, and frequently these were multiple tumors. Of our 4 mice with primary malignant ovarian tumors, all had tumors elsewhere; two mice having each two carcinomas of the mammary gland, one having a single carcinoma of the mammary gland, and one a papillary adenoma of the lung which had invaded a blood-vessel, being therefore presumably malignant. The additional tumors in the 26 mice with both ovarian and other tumors, were located as follows:

Sixteen had one or more primary carcinomas of the mammary gland.

Four had papillary adenoma of the lung.

Two had papillary adenoma of the lung and carcinoma of the mammary gland.

One had adenoma of the mammary gland.

One had a carcinoma and a sarcoma of the mammary gland.

One had a subcutaneous sarcoma.

One had a subcutaneous sarcoma and an osteosarcoma.

#### SUMMARY

Among 22,000 mice of the Slye stock dying natural deaths at all ages were 44 mice with spontaneous primary ovarian tumors not including simple ovarian cysts. Of these, 38 had simple benign solid papillary adenomas, only occasionally with slight cyst formation. One showed a typical papillary cystoma. One had a typical solid teratoma containing a great diversity of tissue elements.<sup>6</sup> Of the 38 cases of solid papillary adenomas, 19, or 50 per cent, were bilateral, so that there were 57 tumors of this class. There were 4 unquestionably primary malignant tumors of the ovary, all showing the "mesothelioma" type of growth characteristic of malignant tumors derived from the sex glands; one of these produced perirenal metastases. There was one other tumor of the same type primary in either the ovary or the adrenal. Two round-cell sarcomas were found that arose either from the ovary or some other organ, while two other sar-

<sup>6</sup> A second case of teratoma has been observed since this analysis was made.

comas had produced secondary growths in the ovary. Of the 44 mice with primary ovarian tumors, 26 had tumors in other parts of the body.

In the literature were found reports of 8 other cases of tumors arising in the ovaries of mice, which exhibited quite the same characteristics as the tumors described in this paper.

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Observations on the Toxicity of Tetra-  
nitromethylaniline (Tetryl), Tetra-  
nitroxylen (T.N.X.), Tetranitraniline  
(T.N.A.), Dinitrodichlorbenzene  
(Parazol), and Metanitraniline \*

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**D**URING the war several problems concerning the toxicity of various explosives or chemicals, used in munitions plants, were referred to the Otho S. A. Sprague Memorial Institute for investigation. The sudden cessation of munitions work terminated these investigations, most of them while incomplete. Some definite observations having been made, it has seemed desirable to make them available, and hence the following synopsis of some of our results is published. As they do not represent work carried to completion, the numerous protocols and much negative and inconclusive work are not presented. Numerous members of the Institute have collaborated in this work, as indicated in the text.

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1. TOXICITY OF TETRANITROMETHYLANILINE (TETRYL), TETRANITROXYLENE (T.N.X.), AND TETRANITRANILINE (T.N.A.)

Drs. W. D. Sansum and Julian H. Lewis administered these substances to dogs and rabbits in doses of grams per kilo of body weight and thereby ascertained their lethal dose, the clinical symptoms they produced, and, on autopsy, their pathological effects. The majority of the tests were made with subcutaneous injections of emulsions in olive oil. Attempts were made to give repeated small doses to dogs by stomach tube, but this method produced vomiting even when the substances were given in milk. Repeated small doses given to rabbits by stomach tube were less toxic than those given by the subcutaneous method. Daily inunctions with tetryl, the most toxic of the group, were made over long periods of time in dogs with no fatal results. A dermatitis soon developed in these cases, but no anemia or other blood changes resulted. In general, these substances were found to be relatively non-toxic, which may be partly explained by their relative insolubility.

*Tetryl*

The smallest fatal dose was 0.5 gm. per kilogram of recrystallized tetryl given to a dog subcutaneously in olive oil as five daily doses of 0.1 gm. per kilogram each. The dog lived fifteen days. Autopsy showed grossly a mild nephritis. Usually the organs showed no distinct gross pathological

changes. The largest non-fatal dose was 2.5 gm. per kilo given subcutaneously as a single dose in olive oil. Five grams per kilo killed in less than eighteen hours. With these large doses much of the tetryl was unabsorbed. Rabbits given 1 gm. per kilogram of body weight by stomach tube in milk were killed after one to three doses. The same amounts given subcutaneously in olive oil were sometimes but not always fatal after six to ten doses.

Microscopically the tissues of rabbits and dogs after fatal doses of tetryl showed at the site of injection a severe acute inflammation, with more or less edema and hemorrhage and sometimes waxy degeneration of muscles lying in the injected material. Without exception the kidneys showed toxic degenerations, so that in animals dying after a few days the epithelium was intensely swollen, with resulting albuminuria; usually there was much protein in the tubules and glomerular spaces, but little tendency to form casts. In one dog, dying within eighteen hours of the time of injection of 5 gm. of tetryl per kilo, the glomerular capillaries were distended with agglutination thrombi of fused red cells. In some cases there were areas of necrosis in the convoluted tubules. Fat granules were frequently found in the epithelium of the ascending loops of Henle in dogs, usually in moderate amounts, with little or no fat elsewhere. In rabbits the fatty changes were not found, although swelling and degeneration of the epithelium was marked.

The liver, in several dogs, showed marked changes, consisting of varying degrees of necrosis in the centers of the lobules and usually severe fatty degeneration of the liver cells, and also a noticeable deposition of fat in the bile duct epithelium. The rabbit livers showed almost no changes. Several of the animals showed more or less edema in the lungs and bronchi, in one case thrombi being observed in the pulmonary artery.

The spleens often showed blood pigment in moderate amounts, but without the congestion usually observed with hemolytic poisons. No noteworthy changes were observed in the other viscera.

#### *Tetranitroxylene*

The smallest fatal dose by subcutaneous injection was 1.2 gm. per kilo given to a dog as twelve daily doses of 0.1 gm. per kilo. The dog lived twelve days. The urine contained albumin and the kidneys showed a parenchymatous swelling. A rabbit receiving 1 gm. per kilo intraperitoneally died during the night, with much ascites. The largest non-fatal dose was 5 gm. per kilo given to a rabbit as a single dose subcutaneously in olive oil. No larger doses were given. The meta and para T.N.X. seemed to have the same toxicity. T.N.X. had no effect on the blood count. Goldfish were killed in fifteen minutes in a 1:100,000 solution of T.N.X., while they lived indefinitely in a 1:1,000,000 solution.

Microscopically the effects of T.N.X. were seen chiefly in the kidneys, in both



dogs and rabbits the epithelium of the convoluted tubules showing more or less vacuolization, but no cast formation. A dog that received ten daily doses of 1 gm. each showed considerable calcification of necrotic tubular epithelium, similar to that seen in mercuric chloride poisoning. There was no excessive pigmentation of the spleen or other evidence of hemolysis. A rabbit that had four daily doses, each of 1 gm. per kilo subcutaneously, showed several areas of focal necrosis in the liver. A rabbit that received nine doses of 1 gm. per kilo by mouth in capsules showed only a fatty degeneration of the epithelium of the collecting tubules of the kidney and no other changes, indicating that little of the T.N.X. was absorbed. Two dogs, given each 5 gm. per kilogram in one dose subcutaneously and dying within eighteen hours, showed extreme fatty degeneration of the ascending limb of Henle's loops, but no other changes. In a few instances a slight fatty degeneration was observed in the center of the liver lobules.

#### *Tetranitraniline*

The smallest fatal dose was 2.5 gm. per kilogram given to a dog subcutaneously as a single dose in olive oil. The dog lived six days. The largest non-fatal dose was 5 gm. per kilo given to a dog as above. No larger doses were given. Rubbed on the skin as an ointment, T.N.A. produced a dermatitis, and apparently some of it was absorbed, because of the effect produced on the urine. T.N.A. had no effect on the blood counts.

Goldfish were able to survive in a 1:12,000 dilution of T.N.A. for at least two weeks.

Microscopically the effects of T.N.A. were as follows: At the point of injection of T.N.A. in oil there occurred a marked acute inflammatory reaction without suppuration. At times we have seen dark yellow masses of the material that have infiltrated ducts of the mammary gland and led to a striking epithelial proliferation, recalling the effects observed when oil solutions of Sudan III and Scarlet R are injected into epithelial tissues. Ordinarily the viscera showed no important gross or microscopic changes in dogs or rabbits given even large or repeated doses of T.N.A. The urine occasionally showed a little albumin, but not usually, and the renal changes were insignificant.

## 2. THE ACTION OF PARAZOL (DINITRO-DICHLORBENZENE) ON THE SKIN, AND SUBSTANCES WHICH WILL PREVENT IT

The experiments were conducted by Dr. W. B. McClure and Dr. H. O. Lussky.

Parazol melts at 60°. The pungent odor of the crude substance becomes more marked as the temperature is raised. At about 125°C. dense white fumes are produced and they become more dense as the temperature increases. These fumes are heavier than air and have a pungent penetrating odor. Crude parazol applied to the shaved skin of a rabbit produces hyperemia, edema, and at times necrosis, the degree varying with the duration of application. (The experiments extended up to two

hours only.) On removing the parazol at the end of the application there is redness and edema extending well beyond the area of direct contact. This redness and edema usually reach their maximum of extent in area and degree at the end of six hours, and have usually subsided considerably at the end of twenty-four hours. The edema next disappears entirely, and finally after about four to six days there is a superficial desquamation, and then complete disappearance of the lesion. Occasionally superficial necrosis occurs. Linseed oil or water added to parazol causes no different reaction. The sublimate obtained by cooling the fumes arising on heating parazol to  $80^{\circ}$  are apparently slightly less active than parazol. In lanolin, parazol seems less active. Melted parazol has about the same toxicity as crude parazol, while purified parazol is distinctly less toxic than crude parazol. Mice lived in cages over crude parazol, apparently without harm. When exposed to fumes from heated parazol they gave evidence of marked skin irritation, but three-minute exposures were not fatal.

Crude parazol applied to the skin of man has the following course of action. Up to one hour application, no reaction occurred. Application for two hours produced hyperemia, finally a small vesicle. Application for three hours and fifteen minutes caused an itching at about three hours. A red slightly raised lesion, larger than the area of contact of parazol, was present on removal of the preparation; the edema increased somewhat during the next seven



hours. After twenty-four hours the lesion was denuded superficially and tender to the touch. No extension of the area occurred. After forty-eight hours a flabby blister containing a clear fluid replaced the red lesion. After four days the vesicle collapsed and the edges were somewhat raised. After six days there was a red-brown scab. On the seventh day appeared what was probably a secondary dermatitis, manifested by secondary vesicles and hyperemia about the lesion, and marked itching. This gradually subsided and scaling resulted in final healing after about seven more days, only a small brown-red discoloration persisting.

Fumes from parazol heated to  $80^{\circ}$  and applied to rabbit's skin for five to fifteen minutes produced only a mild reaction (hyperemia and very superficial scaling with little or no edema). The fumes produced by heating to  $150^{\circ}$  and applied to rabbit's skin for five minutes produced a reaction definitely greater than fumes from the  $80^{\circ}$  heating. The white fumes produced by heating over a free flame and applied to the skin for one to eight minutes produced a much more marked reaction than the other applications.

Rabbit's shaved skin is protected against crude parazol by the following (given about in decreasing order of degree of protective power):

- |               |     |                          |
|---------------|-----|--------------------------|
| 1. Sample C   | (1) | } All having G.A.G. (12) |
| 2. Sample A   | (2) |                          |
| Material Z    | (3) |                          |
| Material Z II | (4) |                          |
| Material IV   | (5) |                          |
| Cloth III     | (6) |                          |

3. Oil silk (7)  
     Water-proof horsehide (8)  
     Oil cloth (9)  
     Adhesive plaster (10)  
     Split leather (11)  
     Glove leather (ordinary dress glove leather)
4. Rubber was a poor protective
1. Sample C = Water-proof cloth. Two layers with 5 layers of G.A.G.
2. Sample A = Same as Sample C but with 2 layers of G.A.G.
3. Material Z = Muslin impregnated with G.A.G. and hardened with chrome salts.
4. Material Z II = Muslin impregnated with G.A.G. three times, then hardened with chrome salts.
5. Material IV = Muslin impregnated with G.A.G. three times.
6. Cloth III = Water-proof paper with 2 layers of G.A.G.
7. Oil silk = Bauer and Black.
8. Water-proof horsehide = Pfeister and Vogel Leather Co., Milwaukee.
9. Oil cloth = Cheap grade.
10. Adhesive plaster = Lewis Manufacturing Co., Walpole, Mass.
11. Split leather = Pfeister and Vogel Leather Co.
12. G.A.G. = (Helmholz and Lussky) glycerol, 50; acacia, 50; glue, 50; water, 200 parts.

### 3. TOXICITY OF METANITRANILINE. ITS QUANTITATIVE DETERMINATION IN THE URINE

These experiments were performed by Dr. Julian H. Lewis.

Metanitraniline is a yellow, light, fluffy, odorless powder. The sample used for experiments had a melting point of  $111.5^{\circ}$  ( $114^{\circ}$  Beilstein). It is soluble in the organic

solvents and slightly soluble in water. It forms a 3.5 per cent. solution when saturated in olive oil. The dry powder is rather volatile, as is shown by the fact that a filter paper covering a dish of the dry substance is colored yellow over night at room temperature. It is toxic for experimental animals, but not markedly so. Its toxicity for the various animals used stands in the following descending order: dog, cat, guinea pig, rabbit. A 6.3 kg. dog was killed in four hours by an intraperitoneal injection of 15 c.c. of a 3.5 per cent. solution in olive oil, or 0.07 gm. per kilo. The average sized rabbit (1.7-2 kg.) will stand an intraperitoneal injection of 15 c.c. of the 3.5 per cent. solution (0.2-0.25 gm. per kilo), without any marked toxic effects, but 20 c.c. usually kills and 30 c.c. always kills. One rabbit (1.9 kg.) received eleven daily injections of 10 c.c. of 3.5 per cent. M.N.A. subcutaneously without serious effects. A 2025 gm. cat was killed with 15 c.c. of the 3.5 per cent. solution given intraperitoneally. A 970 gm. guinea pig was killed with 7 c.c.

Death in all of the animals was acute and was manifested by dyspnea and convulsions. The autopsies of these animals showed the signs of asphyxia. The blood was of a dark color and did not coagulate readily. Methemoglobin was not found in the blood on spectroscopic examination. Rabbits given repeated sublethal doses rapidly developed a profound emaciation and a blood examination of these animals showed a severe secondary anemia. For example, a



rabbit was given subcutaneously 15 c.c. of 3.5 per cent. solution of M.N.A. in oil on December 1. Before the injection the red count was 6,560,000; white cells, 8,560, with 58.5 per cent. polymorphonuclears, 33.3 per cent. small mononuclears and 8.2 per cent. large lymphocytes. On December 3, the counts were: red cells, 3,792,000; white cells, 14,600, with polymorphonuclears 55.9 per cent., small mononuclears 27.1 per cent., large lymphocytes 12.3 per cent., unknown cells 4.7 per cent. The injection was repeated December 3, and on December 8, the red count was 1,548,000, with 6,233 white cells which included so many atypical forms that a differential count could not be made. On autopsy the changes of the bone marrow characteristic of anemia were found. The kidneys and spleen were usually swollen and very dark in color.

Microscopically the anatomical changes produced by M.N.A. were far more marked than those resulting from T.N.A., T.N.X. or tetryl. In animals living a few days there occurred a profound hemolysis and the changes found in the tissues seemed to depend on this hemolysis. The renal tubules were distended with masses of hemoglobin, either as small globules or fused into casts, which sometimes seemed to occlude nearly all the collecting tubules. The tubular epithelium was usually relatively unaffected, at most showing swelling or vacuolization, with little or no necrosis. In some the glomerules showed little or no change; in others the tufts were compressed

by a protein exudate which did not seem to contain hemoglobin. No hemorrhage or inflammatory changes were observed. There was usually fatty degeneration of the epithelium of the straight tubules, especially in Henle's loops, in rabbits as well as in dogs.

In several cases there occurred more or less necrosis of the liver cells in the centers of the lobules, occasionally marked, and there was usually central fatty degeneration of varying degree. Fatty degeneration of the myocardium was sometimes found.

The spleen was distended with red corpuscles in acute cases, and contained much blood pigment in animals living longer. Often the blood in the vessels of preserved sections showed extensive hemolysis, presumably postmortem. Leucocytosis was often conspicuous.

The vessels of the lungs usually, and less often of the liver and other viscera, showed thrombi, sometimes of fibrin and sometimes apparently of agglutinated erythrocytes. This probably accounts for the varying degrees of pulmonary edema and distention of the right heart that were commonly observed, and for the sudden death with dyspnea and convulsions.

The urine of animals injected with toxic doses of metanitriline showed marked and constant changes. It was of a dark reddish-brown color. It contained a trace of albumin and sometimes a few red cells; however, no hemoglobin was demonstrable with the guaiac test or spectroscopically. Methemoglobin was not present. This con-

TABLE 1. — DETERMINATIONS OF METANITRANILINE IN THE URINE OF ANIMALS

Animal	Weight	Dose of Metanitriline	Volume of Urine	Amount of Metanitriline in Urine, Colorimetric Determination
Rabbit	1870 gm.	0.53 gm.	1st day, 100 c.c.	= 0.0675 gm.
			2d day, 110 c.c.	= 0.0053 gm.
			3d day, 17 c.c.	= 0.0 Total, 0.0728 gm.
Rabbit	2130 gm.	0.53 gm.	1st & 2d day, 79 c.c.	= 0.0775 gm.
			3d day, 70 c.c.	= 0.0 Total, 0.0775 gm.
Rabbit	1940 gm.	0.53 gm.	1st day, 85 c.c.	= 0.0612 gm.
			2d day, 94 c.c.	= 0.0213 gm.
			3d day, 109 c.c.	= 0.0 Total, 0.0825 gm.
Dog	10.5 kg.	1.05 gm.	Died in 3 hrs., 150 c.c.	= 0.00087 gm.
Dog	6.3 kg.	0.53 gm.	Died in 4 hrs., 1st hour's urine	= 0.00016 gm.



dition lasted about three days and the urine finally became normal. A second injection of metanitriline always produced much less change than the first injection. In fatal cases there developed anuria, probably from occlusion of the tubules by hemoglobin casts.

Metanitriline could always be demonstrated in the urine after injection, both in the free form and conjugated. To illustrate this, a sample of urine without previous treatment was extracted with ether. The ether was colored yellow. The extraction was continued until the ether was no longer colored. The urine was then mixed with an equal volume of 40 per cent. sulphuric acid, allowed to stand one-half hour, and then neutralized with 20 per cent. sodium hydroxide. On repeating the extraction with ether it was again colored yellow. The extraction was continued until complete. The ether from both extractions was evaporated to dryness. Pure crystals of metanitriline, determined by their melting point, separated on the sides of the vessels.

Dr. M. Th. Hanke devised a colorimetric method for estimating metanitriline, which was based on the method of determining nitrates with paraphenylenediamine. Metanitriline in a weak alcoholic solution is reduced to paraphenylenediamine with zinc dust and sulphuric acid. When mixed with sodium nitrate, paraphenylenediamine gives a brown color (Bismarck brown) which can be estimated colorimetrically. Table 1 gives the results of determinations of meta-

nitraniline in the urine of animals after the injection of this substance. Plans to examine the urine of workers in metanitriline factories to see if metanitriline could be demonstrated by this method were frustrated by the cessation of war.





# The Relations of Proteinogenous Amines to Medicine

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# THE RELATIONS OF PROTEINOGENOUS AMINES TO MEDICINE

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January 30, 1920

## INTRODUCTION

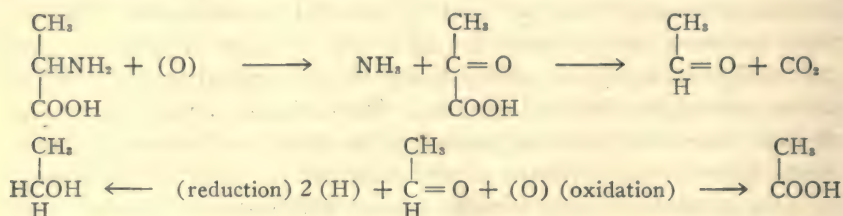
The injurious action which pathogenic micro-organisms exert on the human organism is almost entirely effected by chemical means. Yet our comprehension of the chemical processes by which bacteria produce disease and our knowledge of the chemical constitution of the poisonous substances evolved by their metabolism is in a very elementary state. To the student who would venture to dedicate his life-work to the investigation of the chemical factors in the pathogenesis of infectious diseases, the history of the last thirty years of the Nineteenth Century tells a tale that merits his reflection.

About the time when Pasteur, in France, had communicated his fundamental researches on fermentation and putrefaction, proving that these two processes depended invariably on the presence and activity of living micro-organisms, another chemist, in Italy, Selmi, was occupied in investigating the end-products of putrefaction found in human bodies after death. Selmi observed that basic compounds occurred in the animal organism of similar chemical character as the alkaloids of vegetable origin, giving reactions like those of coniin, nicotin, atropin, delphinin and strychnin. Since he found these putrefactive alkaloids chiefly at the medicolegal examination of corpses, he gave them the name ptomaines, from *πτῶμα* — corpse. Selmi worked entirely with the extracts of the putrefied material and recognized the proper solvents in ether, chloroform and amylalcohol, but he himself never succeeded in isolating a single ptomaine in pure crystalline form. This achievement was accomplished by Nencki, one of the most eminent investigators in the field of chemical bacteriology. Nencki, in 1876, first isolated and analyzed correctly a base of the formula  $C_8H_{11}N$  obtained in the putrefaction of gelatin and identified it later as phenylethylamin. Of the large number of investigators that followed Selmi and advanced this field, Gautier, in France, and Brieger, in Germany, were the most successful. By using new precipitating agents like the chlorids of the heavy metals, as mercuric and platinic chlorid, they

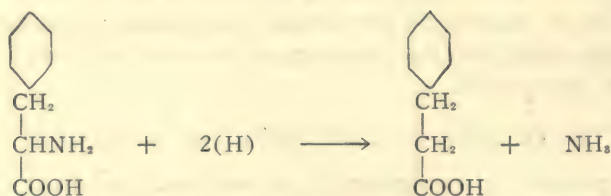


faculty of breaking the amino acids into smaller pieces, and some of these fragments are extremely toxic. There are two main routes over which this katabolism of amino acids may proceed.

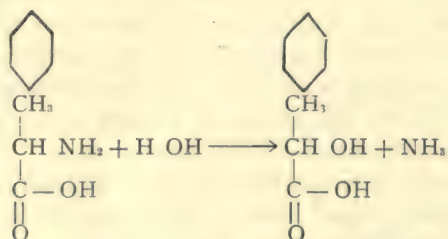
First: The amino group is detached, ammonia is split off and the next lower fatty acid is formed from the amino acid over the keto acid and aldehyd. This process is commonly accomplished by oxydation and is known as oxidative deamination. Thus alanine may be converted into acetic acid:



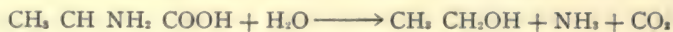
Anaerobic bacteria frequently reduce amino acids; ammonia is liberated and a saturated fatty acid formed. Thus phenylpropionic acid is formed from phenylalanine by this reductive deamination.



In rarer instances the oxyacid is formed from the amino acid by hydrolytic deamination.



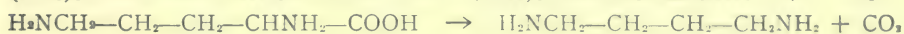
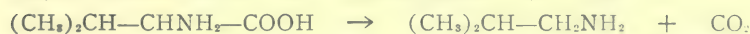
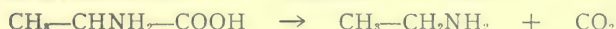
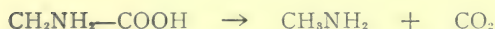
By the activity of yeasts, on the other hand, alcohols are formed from the amino acids by hydrolytic deamination.



Deamination is often followed by decomposition of the carboxylic acid formed, and by oxidation and demethylation. By this series of reactions paracresol and phenol are formed from tyrosin, skatol and indol from tryptophan. These are the compounds we are in the habit of thinking of when we hear the word putrefaction.

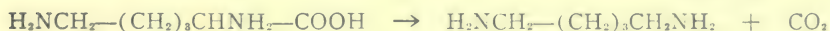
The second main route by which the breakdown of amino acids may proceed is the one to which I particularly wish to direct your attention. The carboxyl group is removed from the amino acid group by the splitting off of carbon dioxid. This process is known as decarboxylation. It may happen before or after deamination. If it precedes the splitting off of  $\text{NH}_3$ , basic substances, amines, of remarkable physiologic properties, are formed, and we define accordingly as proteinogenous those amines that are derived from amino acids by decarboxylation.

In this manner :



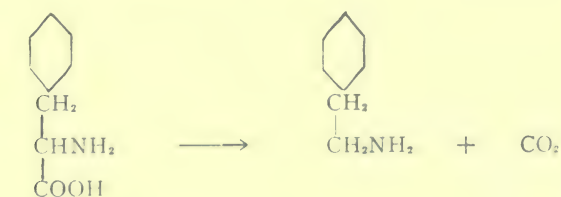
Ornithine

Putrescine



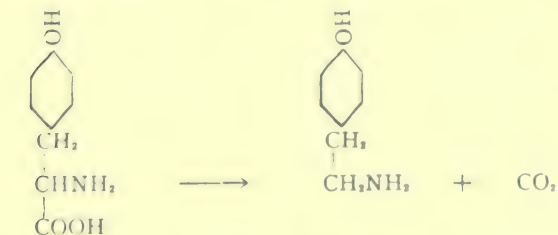
Lysine

Cadaverine



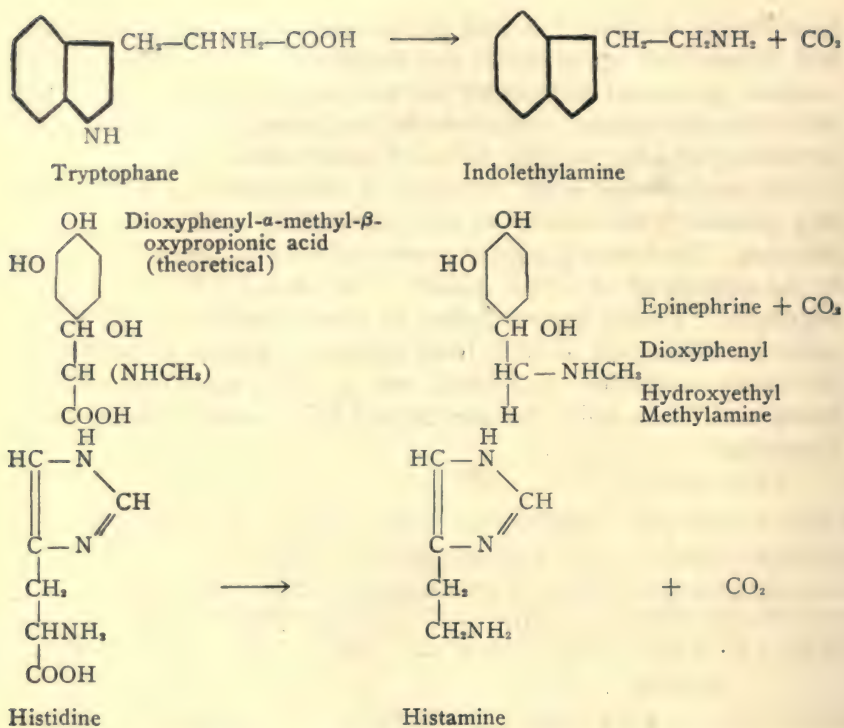
Phenylalanine

Phenylethylamine



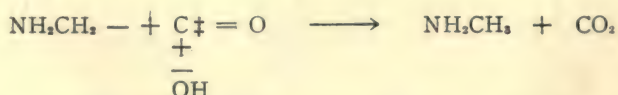
Tyrosine

Tyramine

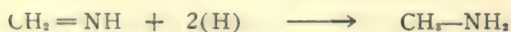
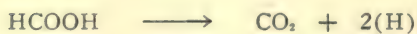
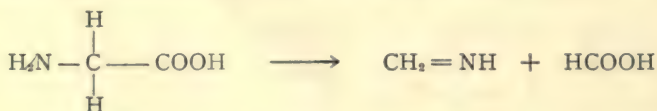


The removal of the CO<sub>2</sub> might be accomplished in various ways.

1. Directly



2. By dissociation into the methylene derivative and formic acid and subsequent reduction of the first to the amine.



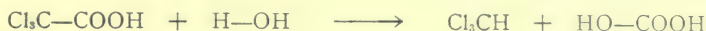
and



## 3. By hydrolysis.



which is similar to the hydrolysis of trichloroacetic acid



This seemingly simple chemical process, the decarboxylation of amino acids to exceedingly potent substances of amine structure is of great theoretical, as well as practical, interest. The relation of this problem to the general nutrition of bacteria, to the metabolism of amino acids in the mammalian organism, to the pathology and pharmacology of the smooth muscle fiber system and to the chemical constitution of the glands of internal secretion, stamps it as a fundamental inquiry of biology.

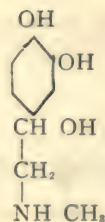
The systematic investigation of certain phases of these problems has been under way in the Sprague Institute for Medical Research for several years. But, before referring to our own work, I wish to discuss briefly the truly remarkable physiologic action of these amines.

## PHYSIOLOGIC AND PHARMACODYNAMIC ACTION

From the point of view of chemical composition they might be divided into two classes: the mono amines, like isobutylamine, isomylamine phenylethylamine, p. hydroxyphenylethylamine and indol-ethylamine, which have one basic N group; and the diamines, as tetramethylene diamine and pentamethylene diamine (putrescin and cadaverin) agmatine, the amine derived from arginine, and imidazole-ethylamine, which have two basic N groups. This difference in the chemical composition of each group is associated with a corresponding diversity of physiologic action. The most active compounds are those that have a ring structure with a side chain of two carbon atoms. As representative of the monoamines, p. hydroxyphenylethylamine will serve for our discussion. P. hydroxyphenylethylamine is derived from tyrosine by loss of carbon dioxide and is therefore also called tyramine. When injected intravenously in quantities of 1-2 milligrams into cats, dogs and rabbits it causes a sudden marked rise in blood pressure. This rise in blood pressure is pronounced and inferior only to the one produced by epinephrine, to which tyramine bears a striking similarity both pharmacologically and structurally.



Tyramine



Epinephrine

While the rise in blood pressure of p. hydroxyphenylethylamine is lower and slower than that of epinephrine, its duration is somewhat longer. Dale showed that the rise of the arterial pressure is partly due to an increased output of the heart, chiefly, however, to a vaso constrictor effect on the arterioles, peripheral in origin. In its action on the uterus, tyramine too resembles adrenaline; it inhibits the activity of the nonpregnant uterus causing relaxation of tonus in the cat, but produces intense contractions of the pregnant uterus. Thus the action on the animal organism produces symptoms which are similar to those produced by stimulation of the sympathetic nervous system. Especially marked is this action on the involuntary musculature of the eye. The intravenous injection of p. hydroxyphenylethylamine in the cat produces dilatation of the pupil, retraction of the nictitating membrane, widening of the palpebral fissure (lagophthalmus), protrusion of the eyeball (exophthalmos), and secretion of tears. Even after extirpation of the superior cervical ganglion these effects are still produced and they must therefore be considered peripheral in origin like similar effects of adrenaline. Of effects on gland cells, beside the lacrimal secretion, we might mention further the action on the sweat glands and submaxillary glands, sweat and saliva being secreted profusely. The flow of urine from the ureters is distinctly increased, but the pancreatic secretion is not visibly influenced and no glycosuria is produced even if as much as 100 mg. are given hypodermically. Nor does parahydroxyphenylethylamine show the dilatating effect on the smooth muscle fiber system of the bronchi, so characteristic of the action of epinephrine. Another important difference in the action of the two bases is shown in their mode of absorption by the organism. The action of epinephrine is almost entirely lost if given by mouth, while the tyramine retains its activity by this channel of absorption, producing all the symptoms of stimulation of the sympathetic nervous system in a marked degree.

The action of *p.* hydroxyphenylethylamine on the circulation in man is comparable to that in animals. If injected subcutaneously in doses from 0.02 to 0.06, it causes considerable elevation of blood pressure lasting for several hours. The increased systolic pressure is associated with a heightened pulse pressure and an increase of the volume pulse in the arm; the heart rate is retarded. In the electrocardiogram the ventricular complex shows an increase in the size of the T wave and the occasional occurrence of extra systoles (Hewlett):

The physiologic activity of parahydroxyphenylethylamine was first revealed by Barger and Walpole, who recognized it as the chief pressure principle in extracts of putrid meat along with isoamylamine and phenylethylamine, which have a similar though lesser action.\* On inoculating a culture medium of tyrosin with a small amount of human feces, Barger and Walpole were able to extract a substance which in the animal experiment behaved like parahydroxyphenylethylamine. This result induced them to express the probability that the amines isolated from putrid meat (parahydroxyphenylethylamine, phenylethylamine, and isoamylamine) are normally formed by putrefaction in the human intestine. Preceding this work of Barger and Walpole on the pressure principles of putrid meat, Dixon and Taylor had made the interesting observation that alcoholic extracts of the human placenta contained substances which on intravenous injection produced a rise of blood pressure and contraction of the pregnant uterus. But it was soon shown by Rosenheim that this effect was given only when the placentae had undergone putrefactive changes, as evidenced by the presence of micro-organisms. Dixon and Taylor had used an amount of alcohol, which, when mixed with the water content of the placenta, was insufficient to prevent putrefactive processes. When perfectly fresh placentae with sufficient absolute alcohol were used, no blood pressure raising substances could be extracted. The active substances were later identified as parahydroxyphenylethylamine and isoamylamine.

When parahydroxyphenylethylamine is given either by intravenous injection or by mouth for a long period of time, the effects of this slow, chronic poisoning are remarkable. Harvey fed and injected rabbits with gradually increasing doses of a 2 per cent. solution of tyramine,

\* The discovery that extracts of putrid meat raise arterial blood pressure on intravenous injection is usually ascribed to Abelous, who reported on this subject in 1906. But 50 years earlier P. L. Panum had discovered and described this fact showing that the poisonous substance retained its activity after boiling for 11 hours and after treatment with absolute alcohol.



over a period varying from 80 to 300 days. At the postmortem examination he found that 20 out of 33 animals showed renal lesions; in 25 there were lesions of the tunica media of the arteries; in 10 animals the enlarged heart showed fibrous changes. The daily subcutaneous injection of 3 mg. of parahydroxyphenylethylamine over a period of 2 weeks produces a severe anemia which leads to the death of the animal. The morphologic changes in the blood, as well as in the bone marrow, recall vividly the picture of true pernicious anemia in man (Iwao).

It might be mentioned that besides putrefied meat, other protein-containing material in a state of slow decomposition has been shown to contain the substance under discussion, such as several varieties of cheese: Cheddar and Swiss, and Japanese soja beans.

Another source of parahydroxyphenylethylamine which deserves our attention from different points of view, is ergot. Until about 10 years ago the action of this drug on uterus and blood vessels was ascribed to the presence of several alkaloids, such as ergotoxine, ergotinine, sphacelotoxin, cornutine and others. But as the result of the work of Rielander, Ackerman and Kutscher on the continent, and Barger and Dale in England, it was found that watery ergot extract contained parahydroxyphenylethylamine, isoamylamine, phenylethylamine, *N*-imidazolethylamine, penta- and tetramethylenediamine and agmatine, and that the action of ergot on the uterus and blood vessels could be explained on the basis of the combined pharmacodynamic effect of these amines. It is still an open question whether these bases are produced entirely by the enzymes of the fungus itself, or by bacterial action during the not sterile process of extraction. In either case the notorious variability of the official liquid extract is readily intelligible.

This brings us to the second group of the simple amines derived from proteins: the diamines, which contain two nitrogen groups. To this group belong indolethylamine, the amine derived from tryptophane by loss of  $\text{CO}_2$ , putrescine and cadaverine, agmatine, the amine derived from arginine, and imidazolethylamine or histamine, the base derived from the amino acid histidine. The amine derived from tryptophane indolethylamine regarding its physiologic activity takes an intermediate position between the sympathomimetic monamines as represented by epinephrin or parahydroxyphenylethylamine and the diamines such as imidazolethylamine. The chemical structure shows a beautiful correlation to this physiologic action, for while the molecule of indolethylamine has two nitrogen atoms, only one of these is basic in nature.

We stated that the most active of these compounds are the cyclic amines with a side chain of two carbon atoms. Of the diamines it is the amine derived from histidine ( $\alpha$  amino B imidazolepropionic acid) which shows such a structure. Its chemical name is B imidazolethylamine, but it may be appropriately called by its shorter name histamine. The physiologic behavior of this amine is primarily characterized by its stimulating effect on the smooth muscle fiber system.

If a guinea-pig weighing 300 to 500 gm. receives an intravenous injection of 0.5-1.0 mg. of histamine, the animal shows, a few seconds after the injection, a state of restlessness and exaltation. This is sometimes preceded by violent scratching of the ears, nose, and the skin of the body which can be reached by the paws of the posterior extremities. The guinea-pig, after a few turning movements, sits in a sprawling position; the heart beat is accelerated, and the respiration, first rapid, soon becomes irregular and labored. The animal passes urine and often feces. Suddenly it begins to run as though fleeing from an invisible enemy, then stops, and after several ineffective respiratory efforts, it falls to one side; the visible mucous membranes of the nose and of the genital region show a high degree of cyanosis. After a few veritable respiratory convulsions the animal dies, plainly a death from acute suffocation. At postmortem the lungs are found to be completely and permanently distended by air, they do not collapse, and are of a pale, anemic appearance, and sections show the bronchioles completely obturated as a result of a tetanic constriction of the smooth muscle fibers surrounding them. Atropine possesses a protective action against this death from acute emphysema and bronchospasm. When larger doses of histamine (3 mg.) are given to the guinea-pig intraperitoneally, a gradual fall of temperature from 38.5-28.5 degrees may be observed in the course of two hours. The animal usually recovers, and the following day the temperature is again about 38 degrees (Dale and Laidlaw). Very small doses, on the other hand, may produce a transitory rise in temperature (Pfeiffer, 1911).

In this picture is recognized the close resemblance to the syndrome produced by poisoning with so-called Witte's peptone, with Vaughan's poisonous fraction of the split protein, and to the protein intoxication following the reinjection of previously sensitized animals known as anaphylactic shock. The chief point of difference lies in the lack of coagulability of the blood brought about by the injection of peptone,



which is present only to a slight degree on the injection of histamine. Another point of similarity to anaphylactic shock is shown by the identical behavior of different species of animals to the amine as well as to the anaphylactic poison. Dogs show a much greater resistance to both forms of poisoning, the chief symptom being a gradually increasing excessive fall of the arterial blood pressure. The possible relation of histamine shock to surgical or traumatic shock is suggested by the study of the effect of this amine on the circulation in the anesthetized cat (Dale). The nonanesthetized cat tolerates large doses; it goes into collapse and coma, but recovers. If the same animal is put under an anesthetic "even moderate doses of histamine will produce a fatal circulatory collapse and respiratory failure, from which the animal does not recover even after prolonged application of artificial respiration." Analysis of this shock shows that the diminution of the output of the heart almost to the point of extinction is the principal factor of the circulatory collapse. This failing systolic output from the heart is due to inadequate filling during the diastole, the greater part of the blood collecting in the capillaries of the voluntary muscles. The fall in blood pressure is very marked, in some cases from 160 to 40 millimeters mercury; the blood viscosity, the corpuscular content and hemoglobin increase, due to loss of blood plasma and its passage into the tissues. But this "leakage of plasma from the vessels into the tissues, with the reduction in the volume and increase in the viscosity of the blood, cannot be the main cause of the shock, though it doubtless accentuates its severity. The characteristic features of the condition are not so much due to the fact that the volume of the blood is reduced, as to the tendency of what remains to stagnate in the periphery in the capillaries and venules instead of returning to the heart; this peripheral accumulation of blood is the effect of a general relaxation of the capillary vessels."

That chemical factors might be involved in the production of traumatic shock has been made probable by the work of Bayliss and Cannon, who showed that on extensive injury to the tissues of a limb, the nervous connection of which with the rest of the body has been severed, shock results; but if the blood vessels are clamped, shock is prevented. They reason that through the injury of the tissues substances are produced which on absorption into the general circulation, produce shock.



The action of histamine on the organs of the smooth muscle fiber system is best illustrated by its faculty to contract the smooth muscle fibers of the nonpregnant uterus in as small a concentration as 1:25,000,000. On gland cells histamine has a stimulating action eliciting on intravenous injection increased secretion of tears, of saliva, of bronchial mucus, and of gastric and pancreatic juice. If a drop of histamine solution in concentrations of from 1:1,000 to 1:100,000 is applied to the scarified skin, it produces within one minute, itching, reddening, and finally a large urticarial wheal which sends out pseudopodia-like processes and measures from 2.5-7 cm. in diameter. These wheals feel hard and resistant, are movable with the skin and cannot be removed by pressure. Subcutaneously, in doses from 0.5 to 1 mg., it produces erythema of the entire skin.

#### RELATION TO PHYSIOLOGY AND PATHOLOGY

The short description of the pharmacodynamic action of the two chief representatives of the proteinogenous amines will suffice to suggest the possible relationship these substances may have to human physiology and pathology. To accept this relation as a scientific fact developed on a sound basis without indulging in interesting but fruitless speculation, it is necessary to satisfy several preliminary inquiries.

1. Have these substances ever been found in the animal organism?
2. How are they formed?
3. What is their fate and function?

Before answering the first and most pertinent question, let us consider the second one. These amines were recognized as the result of putrefaction; this means they were formed from protein material by the action of micro-organisms. The bases were isolated from organs or tissues of animals, permitted to putrefy by being exposed in a haphazard way, either before or during experimentation to the ubiquitous micro-organisms, or organic material was for this purpose inoculated with a piece of putrid meat, most frequently with pancreas. Some of the workers in this field, recognizing the relation of the putrefactive amines to the amino acids, subjected a single amino acid to the action of bacteria and in this way gained a clearer insight into the chemistry of the process. The bacteria involved in this process remained obscure. In 1912, Berthelot and Bertrand isolated in pure culture from the human intestine a micro-organism which had all the characteristics of

the bacillus mucosus capsulatus (Friedländer's pneumobacillus), but in addition the faculty of decarboxylating histidine, tyrosine and tryptophane to histamine, tyramine and indolethylamine, and they therefore named this organism *Bacillus aminophilus intestinalis*. In the same year Mellanby and Thwort described a micro-organism belonging to the typhoid-colon group, capable of producing  $\beta$  imidazolethylamine from histidine. Theoretical considerations and preliminary experiments had convinced us five years ago that the ability to decarboxylate amino acids to amines could not be restricted to one or two species of bacteria, but that it might be found to be a fairly common property of micro-organisms provided that the chemical conditions of life were understood which called forth the formation of these basic products. Investigations on this problem, carried out in the Sprague Institute for Medical Research at the University of Chicago, in association with Dr. Hanke, have been published in part. We could show that the colon bacillus will form histamine from histidine only in the presence of an easily available source of carbon, such as glucose or glycerol; that the production of the amine is always coincident with the production of a medium that is distinctly acid, and we expressed the belief that the amine is formed by the micro-organism to neutralize the excess of acidity. It can therefore be accepted as a proved fact that micro-organisms, that have the faculty of forming poisonous amines from amino acids, live in the intestinal tract of man.

Can this decarboxylation of amino acids occur in the absence of micro-organisms?

The depressor action of certain organ extracts had been known for a long time. The work of Popielski had shown that such "vasodilators" could be extracted practically from all tissues. In 1911, Barger and Dale reported that they had succeeded in isolating  $\beta$  imidazolethylamine from the extracts of intestinal mucosa; they showed that Popielski's "vasodilator" contained histamine and also the depressor action of secretin of Bayliss and Starling could be explained by the presence of this base. The authors at first regarded histamine as a normal product of the intestinal mucosa, but later — under the influence of the work of Mellanby and Thwort — they adopted the view that the formation of the base is probably due to the action of bacilli.

This view is permissible, but it is extremely probable that the digestive cells of the mucosa themselves are able to decarboxylate histidine to histamine. It might be stated as a general principle that

any enzymatic activity possessed by bacteria is uniformly the property of some cells of the mammalian organism or that what the bacterial cell can do, the organ cell is able to do. If, therefore, micro-organisms possess a carboxylase activity, it is safe to assume that the multicellular organism too is possessed of this faculty of decarboxylation. In the disorder of protein metabolism known as cystinuria, the two diamines: putrescin and cadaverin, have been repeatedly found in urine and feces. There is no real evidence that in this condition the diamines are formed by the activity of bacteria from the two amino acids, lysin and ornithin (from arginin), and the view is generally held that the diamines, which cystinurics sometimes excrete, are the products of an abnormal protein metabolism. This conception receives very strong support through the studies of Loewy and Neuberg on a patient excreting cystin in the urine. At ordinary times no diamine could be detected in the urine, but when lysin was given to him by mouth, he excreted cadaverin in large quantities, and when arginin was given he excreted putrescin. Yet the absolute proof that the diamino acids were not decarboxylated by the intestinal bacteria, but by the cells of the organism could have been deduced only from the subcutaneous or intravenous administration of the amino acids. Unfortunately, this has not been done. Thus far it has not been definitely proved that sterile extracts or emulsions of organs like the liver harbor an enzyme of the carboxylase type, or that such enzymes are developed during the aseptic autolysis of organs.

The physiological activity of the hypophysis has been a subject of constant investigation since Oliver and Schaefer, in 1895, discovered that extracts of the gland had the power of raising blood pressure. And when it was shown, some years later, that such extracts of the pituitary body had the power of producing strong contractions of the uterus, a new agent was introduced into therapeutics. Ever since the isolation of the active principle of the gland has been attempted by biochemists, and their investigations have shown that the substance behaves like a base, the only precipitant to bring down the active principle being phosphotungstic acid. Fühner, in association with the other chemists of Meister Lucius und Bruening in Höchst am Main, claimed to have isolated four different crystalline substances, all of which showed physiologic activity in varying degree, and Fühner believes that there exist four active principles. His work, however, invites the

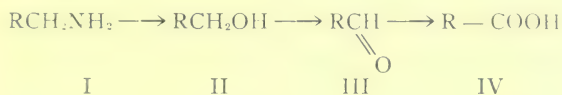


• interpretation that these four active fractions owe their activity on the uterus to contamination, absorption or chemical combination with one and the same true active substance of exceedingly high potency.

The base precipitated from pituitary extracts gives the chemical reactions which characterize it as an imidazol derivative, and since B imidazolethylamine or histamine produces powerful contractions of the uterus, it has been considered quite possible that the active principle of the hypophysis is a polypeptid-like derivative of this base. In June, 1919, Abel and Kubota of Johns Hopkins, published a remarkable paper in the *Journal of Pharmacology and Experimental Therapeutics*, entitled: "On the Presence of Histamine (B iminazolethylamine) in the Hypophysis-Cerebri and other Tissues of the Body and Its Occurrence Among the Hydrolytic Decomposition Products of Proteins." In this communication the authors report the isolation of histamine from the hypophysis, and they consider it the plain muscle stimulating and depressor constituent of the posterior lobe of the hypophysis. They further claim to have demonstrated histamine in the gastric and intestinal mucosa, in the liver and skeletal striated muscle; having found it in Witte's peptone, they conclude that peptone shock is due to histamine and the authors are confident that the toxic principle of Vaughan's protein poison too is histamine. But this is not all of their claim. Convinced that histamine is one of the products of the enzymatic digestion of protein-containing foods, the authors make the extraordinary statement that they have obtained histamine on the acid hydrolysis of egg albumin, casein and edestin. To quote from their paper: "It is our opinion that this substance makes its appearance wherever living protoplasm exists, or at least wherever protoplasm is killed; in other words, that it arises wherever true protein is even partially disrupted by enzymes, acids or other hydrolytic agents." While such statements concerning the great importance of the amines coming from one of the foremost scientists of our country is extremely gratifying to us, who have worked on this subject now for nearly five years, we feel — on the basis of our work\* — more than a little hesitancy in accepting Abel's results in their entirety. But this is not the time nor the place to debate differences of results due to differences in methods. We are in accord in our views regarding the fundamental significance of the proteinogenous amines for the physiology and pathology of the human organism. Regarding the fate of the protein-

\* See our papers in the *Journal of Biological Chemistry*, 1920.

ogenous amines in the animal organism, we are still insufficiently informed. It could be shown by Ewins and Laidlaw that if p. oxyphenylethylamine is given by mouth to dogs, about 25 per cent. is eliminated in the urine as oxyphenylactic acid. It is known that the liver is the organ chiefly involved in this process of deamination and oxydation, for on perfusion of the surviving rabbits' liver, up to 70 per cent. of the theoretically possible acid was actually isolated. Similarly, indolethylamine is converted into indoleacetic acid on perfusion through the liver. The fate of B imidazolethylamine in the organism is still unknown. This katabolism of the amines to a fatty acid proceeds probably by way of the alcohol and the aldehyde according to the following scheme:



By these transformations, these physiologically active substances are detoxicated, in the healthy organism, in the liver. Morbid conditions, then, will depend on the quantitative relationship of poisons produced and detoxicating function. The insufficiency of the detoxicating apparatus or an excessively large overproduction of amines might produce disease.

These are, in brief, some of the newer facts which the study of the proteinogenous amines has established. Their application to the more practical problems of clinical medicine, based on the use of the exact methods developed, promises a rich field. But I have deliberately abstained in my discussion from leaving the firm soil of the evidence gained by experiments to venture out on the perilous sea of supposition and speculation.

*Reprinted from the Proceedings of the Institute of Medicine at Chicago  
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AMERICAN MEDICAL ASSOCIATION  
FIVE HUNDRED AND FORTY FIVE NORTH DEARBORN STREET  
CHICAGO





## STUDIES ON PROTEINOGENOUS AMINES.

### VI. THE PREPARATION OF HISTIDINE FROM BLOOD CORPUSCLE PASTE.

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Since Fränkel<sup>1</sup> published his method for the preparation of histidine, small quantities of this substance have been obtainable without difficulty. The details given by Fränkel are rather incomplete. Most laboratories have found it necessary, therefore, to supply the exact details in order that their students could prepare this amino-acid successfully. One very good unpublished modification with which we are familiar is that devised by Dr. F. C. Koch of the Department of Physiological Chemistry at the University of Chicago. Jones,<sup>2</sup> who was formerly one of Koch's students, has recently published a method for preparing histidine that is essentially that of Koch.

Anyone who has prepared histidine repeatedly by one of these processes cannot fail to have been struck by the uncertainty of the results. The yield of histidine dichloride may vary between the wide limits of 10 to 25 gm. from 2 liters of the same corpuscle paste. Frequently the presence of impurities in the crude histidine dichloride makes it difficult to obtain a good yield of the pure substance. The most serious causes of difficulty seem to be the following.

1. The precipitation of humin and ferric hydroxide by means of sodium carbonate is very unreliable. Even an experienced chemist may waste considerable time before the correct condition for complete precipitation is obtained. By using lime in place of sodium carbonate this difficulty can be removed entirely.

<sup>1</sup> Fränkel, S., *Monatsh. Chem.*, 1903, xxiv, 230.

<sup>2</sup> Jones, H. M., *J. Biol. Chem.*, 1918, xxxiii, 429.

2. The mercury salt of histidine has usually been prepared by adding sodium carbonate and mercuric chloride alternately until precipitation has ceased. We have found that *the precipitation is always incomplete unless an excess of mercuric chloride is present from the start*; that it is necessary to use far more mercuric chloride than has usually been employed; and that by using exact weights of mercuric chloride and sodium carbonate a uniformly complete precipitation can always be obtained.

3. The final purification of the histidine dichloride by the previously described methods has been subject to considerable loss both of time and material. By recrystallizing the crude product from aqueous alcohol under exactly defined conditions, a very good yield of a perfectly pure final product is obtained. The process to be described has given an average yield of 15 gm. of pure histidine dichloride from 500 cc. of blood corpuscle paste, which is about four times the quantity that has usually been obtained.

#### EXPERIMENTAL.

1. *Hydrolysis*.—Fresh blood corpuscle paste,<sup>3</sup> 500 cc., is mixed with 1,000 cc. of 37 per cent hydrochloric acid in a weighed 3,000 cc. long necked, round bottomed Pyrex flask and hydrolyzed by boiling, under a reflux condenser, for 30 hours over an electrically heated asbestos bath.

2. *Removal of the Hydrochloric Acid*.—The hydrochloric acid is removed by distillation *in vacuo* at 60° from the same flask. The residue is finally dried at 100° for 2 hours to remove the water and acid as completely as possible. The flask is weighed and the weight of the residue—usually about 350 gm.—is obtained by difference. This weight is very important because the quantities of mercuric chloride and sodium carbonate used later in the preparation depend upon it. This will be referred to later as Residue 2.

3. *Removal of Ammonia*.—Residue 2 is dissolved in 1,000 cc. of water. The solution is treated with commercial finishing lime until the reddish brown precipitate formed at first assumes a homogeneous clay color due to the presence of undissolved lime.

<sup>3</sup> The blood corpuscle paste was obtained from Armour and Company, Chicago, Ill.

The saturation with lime is necessary to insure a complete precipitation of the ferric hydroxide and humin. The mixture is treated with 500 cc. of 95 per cent alcohol and subjected to a distillation *in vacuo* at 40° until about 800 cc. of distillate have been collected. This removes the ammonia completely.

4. *Removal of Humin and Ferric Hydroxide.*—The mixture is filtered, on a 6 inch Buchner funnel, from humin, ferric hydroxide, and excess lime, the precipitate being carefully washed with a large volume—2,000 cc.—of a hot saturated aqueous solution of lime. The clear amber-colored liquid, which contains all the amino-acids as lime salts, is always free from iron when enough lime has been added.

5. *Isolation of Tyrosine and Leucine.*—The alkaline filtrate obtained under Section 4 is diluted to 4,000 cc., heated on the water bath, and treated with 350 gm.<sup>4</sup> of solid anhydrous sodium carbonate. The resulting mixture is agitated until all the sodium carbonate has passed into solution. This precipitates the calcium as calcium carbonate. The mixture is filtered promptly on a 6 inch Buchner funnel and the precipitate is washed with 1,000 cc. of hot water. The filtrate, which should be free from calcium, is transferred to a 6 liter flask, cooled under the tap, and treated with 37 per cent hydrochloric acid *until the liquid reacts neutral to litmus paper*. Glacial acetic acid is then added until effervescence ceases. The solution is subjected to a distillation *in vacuo* at 50–60° until its volume has been reduced to about 800 cc. Sodium chloride crystallizes out toward the end of the distillation together with small quantities of tyrosine and leucine. The mixture is placed in the ice chest for 4 days which separates the tyrosine almost completely, and considerable leucine. It is then filtered on a 5 inch Buchner funnel, the precipitate being washed with 200 cc. of ice-cooled water.

The precipitate contains about 50 gm. of leucine and 1.5 gm. of tyrosine together with a large quantity of sodium chloride. The

<sup>4</sup> The weights of mercuric chloride and sodium carbonate given here are correct only when the weight of Residue 2 (p. 522) is 350 gm. The weight of this residue is, of course, dependent upon the quality of the blood corpuscle paste. In case Residue 2 does not weigh approximately 350 gm., the quantities of  $\text{HgCl}_2$  and  $\text{Na}_2\text{CO}_3$  to be used can be obtained by proportion.



separation of the leucine from the tyrosine and the further purification of these two amino-acids can be most easily accomplished by the method of Habermann and Ehrenfeld.<sup>5</sup>

The filtrate, which contains the histidine, is diluted to exactly 2,000 cc. It will be referred to as Filtrate 5.

6. *Precipitation of the Mercury Compound of Histidine.*—The filtrate from the tyrosine and leucine—Filtrate 5, volume 2,000 cc.—is divided into four equal parts.<sup>6</sup> Each 500 cc. portion is transferred to a 6,000 cc. flask and diluted with 1,500 cc. of water. The further discussion will be limited to *one* of these portions. The other three portions are treated in a manner identical to that which will be described.

Solid mercuric chloride—350 gm.,<sup>4</sup> *four times the weight of Residue 2 which is present in this portion*—is added to the acid liquid. The mixture is heated on the water bath until the sublimate has passed into solution. A small quantity of a gray to brown flocculent precipitate is always present. This can be more advantageously removed later. The liquid is cooled. The mercury salt remains in solution. A solution of sodium carbonate, containing 70 gm.<sup>7</sup> of anhydrous salt dissolved in 3,000 cc. of water, is carefully added to the above liquid. This precipitates the mercury salt of histidine in the form of a flocculent white solid that settles readily leaving a clear supernatant liquid. A test portion of this liquid should give no immediate precipitate when it is treated with a sodium carbonate solution. The clear supernatant liquid is removed as completely as possible by means of a glass siphon. Distilled water—about 4,000 cc.—is poured into the flask, the mixture thoroughly agitated, and the precipitate allowed to settle. The supernatant liquid is siphoned off as before. The mercury salt is washed seven times in this manner.

7. *Isolation and Purification of Histidine Dichloride.*—The four batches of mercury salt obtained under Section 6 are combined in a 6 liter flask. Hydrochloric acid—37 per cent—is then added until

<sup>5</sup> Habermann, J., and Ehrenfeld, K., *Z. physiol. Chem.*, 1902-03, xxxvii, 18.

<sup>6</sup> This division of material was necessary because of the limited size of the laboratory glassware. Then too, the final volume of the entire portion would be 20 liters which is too large a quantity to be handled easily.

<sup>7</sup> In general use 20 gm. of anhydrous sodium carbonate for every 100 gm. of mercuric chloride.

all the white solid has passed into solution. A small quantity of a gray to brown flocculent precipitate is always left that will not dissolve in hydrochloric acid. The mixture is filtered through a large folded filter paper into a 2 gallon bottle. The pale yellow filtrate is saturated with hydrogen sulfide under pressure which removes the mercury completely. The resulting mixture is filtered on a 6 inch Buchner funnel, and the clear *colorless* filtrate is subjected to a vacuum distillation at 60° in a weighed flask. The resulting pale yellow, exceedingly stiff gum is freed from water and hydrochloric acid as completely as possible by heating *in vacuo* at 80° for 2 hours. The gum so obtained—60 to 65 gm.—is dissolved in 60 cc. of 37 per cent HCl by heating on the water bath. The resulting pale brown solution should be free from crystalline matter<sup>8</sup> and will usually remain clear for days if it is kept at room temperature. A few crystals of histidine dichloride are added and the sides of the vessel scratched with a glass rod. The crystallization of histidine dichloride is immediate and so copious that the mass sets to the consistency of paste in the course of 10 minutes. The mixture is placed in an ice bath where it is kept for 2 days to complete the precipitation of histidine dichloride; it is then filtered on a 3 inch Buchner funnel.<sup>9</sup> The white granular powder is washed first with about 50 cc. of cold 37 per cent HCl and then with a cold mixture containing 20 cc. of 37 per cent HCl and 20 cc. of alcohol. After drying at 100° for 10 hours, this solid, which is nearly pure histidine dichloride, weighs from 17 to 19 gm. The following concrete example illustrates the further purification of this product.

Of the crude histidine dichloride, 30 gm. are dissolved in 20 cc. of hot water. Hot 95 per cent alcohol—200 cc.—is added to the above aqueous solution. The liquid is heated on the water bath until the alcohol boils,<sup>10</sup> when it is filtered through a small

<sup>8</sup> A slight crystalline residue is usually sodium chloride which indicates that the mercury compound of histidine was not washed sufficiently. This inorganic matter can be more advantageously removed later.

<sup>9</sup> It is best to use two thicknesses of a fairly hard but not too retentive grade of filter paper in this case.

<sup>10</sup> When the entire process has been properly conducted, the aqueous alcoholic solution will contain no crystalline residue. If such a residue is present it consists of inorganic salts. These are then removed by the filtration that is carried out in any case to remove shreds of filter paper and bits of broken glass.

folded filter into a 300 cc. Pyrex flask. The clear, nearly colorless filtrate slowly deposits large colorless plates of histidine dichloride. To hasten the crystallization these first crystals are titrated with a glass rod. The mixture is allowed to crystallize for 24 hours in the ice chest after which it is filtered with suction on a platinum cone, the crystals being washed with 50 cc. of cold 95 per cent alcohol. The pure white product so obtained, after drying *in vacuo* over sulfuric acid for 48 hours, is 100 per cent pure histidine dichloride.<sup>11</sup> The first crop weighs from 21 to 22 gm. A second crop—about 6 gm.—of equally pure material can be obtained from the mother liquor from Crop 1 by repeating the above recrystallization with the same proportions of water and alcohol.

<sup>11</sup> For a typical analysis see Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 502.







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### VII. THE QUANTITATIVE COLORIMETRIC ESTIMATION OF HISTIDINE IN PROTEIN AND PROTEIN-CONTAINING MATTER.

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#### INTRODUCTION.

#### *The Relation of Imidazole Derivatives to Certain Biochemical Problems.*

The discovery of the imidazole nucleus in histidine gave rise to considerable speculation as to the possible mode of formation of this heterocyclic ring in living matter. The first suggestion as to its mode of formation was obtained from the then well known discovery by Debus<sup>1</sup> that glyoxal condenses with ammonia and formaldehyde to give imidazole. Windaus and Knoop<sup>2</sup> proved in 1905 that methyl imidazole is formed when glucose is allowed to stand for some time in the presence of zinc ammonium hydroxide. The work of these authors leaves little doubt that the methyl imidazole is formed by a condensation of methyl glyoxal and formaldehyde with ammonia, the two aldehydes having been previously formed from the glucose by the action of the ammonia. It is hardly necessary to say that the above condensations, occurring as they do only in the presence of a high concentration of ammonia, can scarcely be claimed to approach the conditions as we find them in living matter. The synthesis of imidazole derivatives by protoplasm may, nevertheless, involve building stones similar to those employed in the laboratory.

<sup>1</sup> Debus, H., *Ann. Chem.*, 1858, cvii, 204.

<sup>2</sup> Windaus, A., and Knoop, F., *Ber. chem. Ges.*, 1905, xxxviii, 1166.



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Although histidine seems to be more abundant, in living matter, than any other imidazole derivative, there are others that may prove to be quite as important. Among these we might mention carnosine ( $\beta$ -alanyl-histidine<sup>3</sup>); histamine<sup>4</sup> ( $\beta$ -imidazolylethylamine), a substance that has recently attracted considerable attention because of its powerful physiological activity; the purines, that can be considered as condensation products of the imidazole with the pyrimidine ring; the hydantoins; the closely related glycoeyamidines; and creatinine. The last three of the above compounds, although they do not contain a typical imidazole nucleus, have a nuclear cyclic structure like that of the imidazoles. It is possible that a genetic relation exists between all these imidazoles; but this problem as well as the fate of the imidazoles in the animal organism, their relation to the secretagogues and the physiological action of organ extracts, the rôle they play in the diazo reaction of the urine, and many other related questions, could not be successfully approached as long as we were not in possession of an exact, simple, and rapid method for the estimation of imidazole derivatives.

### *The Method in Brief.*

Histidine has been determined in the past by the original method of Kossel and Kutscher,<sup>5</sup> by one of the numerous modifications of this method,<sup>6</sup> and by the group method of Van Slyke.<sup>7</sup> The present method, although it may be no more accurate than the best of those referred to above, has the advantages of being simple, rapid, and direct. The principles involved can be summarized briefly as follows:

<sup>3</sup> Baumann, L., and Ingvaldsen, T., *J. Biol. Chem.*, 1918, xxxv, 263.

<sup>4</sup> See Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 539, for a discussion of previous work on histamine; and Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1920, xliii, 543, for a method of estimation of this substance in complex mixtures.

<sup>5</sup> Kossel, A., and Kutscher, F., *Z. physiol. Chem.*, 1901, xxxi, 165.

<sup>6</sup> Kossel, A., and Patten, A. J., *Z. physiol. Chem.*, 1903, xxxviii, 39. Steudel, H., *Z. physiol. Chem.*, 1903, xxxvii, 219. Kossel, A., and Pringle, H., *Z. physiol. Chem.*, 1906, xlix, 318. Osborne, T. B., Leavenworth, C. S., and Brautlecht, C. A., *Am. J. Physiol.*, 1908, xxiii, 180.

<sup>7</sup> Van Slyke, D. D., *J. Biol. Chem.*, 1911-12, x, 15.

The material is hydrolyzed by boiling with hydrochloric acid. The acid and the volatile phenols, if such are present, are removed by distillation *in vacuo*, after which ammonia and humin are removed by treatment with lime. The material is then divided into two fractions by means of phosphotungstic acid. The phosphotungstate *precipitate* contains the *histidine* together with arginine, lysine, and cystine. Of these four amino-acids, histidine is the only one that gives an orange-red color with an alkaline solution of *p*-phenyldiazoniumsulfonate. Tyrosine, which is, to the best of our present knowledge, the only substance that is normally present in non-putrid, protein-containing matter that could interfere with the colorimetric estimation of histidine, is *not* precipitated by phosphotungstic acid from a dilute solution. The phosphotungstate precipitate is treated with water and sufficient 3 N NaOH to give a clear solution. Histidine is then estimated colorimetrically in this liquid, using the method previously described by us,<sup>8</sup> which is a modification of the familiar qualitative Ehrlich diazo reaction.

Histamine and tyramine, both of which give a pink color with *p*-phenyldiazoniumsulfonate, are also precipitated by phosphotungstic acid. If present they would be estimated as histidine. These amines are, however, never present in non-putrid, protein-containing matter in sufficient quantity to have the slightest effect upon the results.

The method depends, therefore, not upon the actual isolation of histidine, which is time-consuming and subject to the possibility of considerable error, but upon the quantitative determination of the imidazole ring.

#### *Detailed Description of the Method.*

The general mode of procedure can be summarized in the following six steps.

1. *Hydrolysis*.—The protein—1 to 3 gm.—is mixed with 60 cc. of 20 per cent hydrochloric acid in a 400 cc. long necked, round bottomed flask and hydrolyzed by boiling for 28 hours over an electrically heated sand or asbestos bath.

2. *Removal of the Hydrochloric Acid and Volatile Phenols*.—The hydrochloric acid and the volatile phenols are removed by

<sup>8</sup> Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 497.

distillation *in vacuo* at 60° from the same flask. The residue is finally dried *in vacuo* at 80° for 1 hour to remove the last traces of the free hydrochloric acid.

3. *Removal of Ammonia.*—The residue is dissolved in 100 cc. of water and the solution treated with an excess of lime and 50 cc. of 95 per cent alcohol. The ammonia, alcohol, and some of the water are then removed by distillation *in vacuo* at 40° (again from the same flask).

4. *Removal of Humin.*—The mixture is filtered, on a Buchner funnel, from humin and excess lime, the precipitate being carefully washed with a large volume of *hot* water until the washings give a negative Pauly reaction.

5. *Preparation of the Phosphotungstates.*—The alkaline filtrate is acidified by adding a slight excess of hydrochloric acid. The resulting clear, amber-colored liquid is then evaporated to dryness on the water bath in a glass dish. The residue is transferred to a 300 cc. Pyrex Florence flask with a solution of 18 cc. of 37 per cent hydrochloric acid in enough water to give a total volume of 100 cc. The resulting solution is heated on the water bath. To it are then added 100 cc. of a hot 15 per cent aqueous solution of phosphotungstic acid. The resulting mixture is digested for  $\frac{1}{2}$  hour on the water bath. It is then allowed to cool slowly to room temperature, after which it is placed in the ice chest for 48 hours<sup>9</sup> and finally cooled in an ice bath for 24 hours. Phosphotungstates have usually been precipitated at room temperatures. Histidine phosphotungstate is far more soluble at 20° than it is at 0° (see experimental part). By conducting the precipitation at 0°, 0.00571 gm. of histidine remains in solution in 200 cc. of precipitation liquid; a definite quantity that can be accurately corrected for. This quantity will be referred to later as the *solubility correction blank*.

6. *The Colorimetric Determination of Histidine.*—The ice-cold mixture obtained under Section 5 is filtered with the aid of suction, two thicknesses of filter paper and a platinum cone being used in preference to the Buchner funnel process described by Van Slyke.<sup>7</sup> The precipitate is washed freely with an ice-cold liquid containing 18 cc. of 37 per cent hydrochloric acid and 15 gm. of phosphotungstic acid in a total aqueous volume of

<sup>9</sup> Up to this point the method is essentially that of Van Slyke.<sup>7</sup>



200 cc., the liquid having been previously saturated with histidine phosphotungstate. Since the wash liquid is already saturated with histidine phosphotungstate it can dissolve none of this substance from the precipitate. No correction, therefore, need be made for the solubility of the precipitate in the wash fluid.

The well washed precipitate finally obtained is transferred, with the paper, to a 1,000 cc. lipped beaker. Enough of a 3 N sodium hydroxide solution is then added to dissolve the precipitate, a large excess of alkali being carefully avoided. The mixture is filtered through a folded filter paper into a 1,000 cc. volumetric flask, and the beaker and filter pulp are thoroughly washed out with distilled water. The clear, pale yellow filtrate is finally diluted to 1,000 cc. This solution will be referred to later as the *test liquid*. The colorimetric determinations are performed on this liquid according to the method previously described by us.<sup>8</sup> The sodium phosphotungstate, which is always present in this liquid, does not interfere with the color production.

The following results have been obtained by using this method.

*Histidine Content.*

	Colorimetric.	Other methods.
	<i>per cent</i>	<i>per cent</i>
Casein.	2.84 first determination.	2.09 Van Slyke, using the method of Osborne, Leavenworth, and Brautlecht with preliminary phosphotungstic acid precipitation at room temperature (Van Slyke, D.D., <i>J. Biol. Chem.</i> , 1913-14, xvi, 533).
		2.30 Van Slyke, direct precipitation with AgNO <sub>3</sub> and baryta (Van Slyke, D.D., <i>J. Biol. Chem.</i> , 1913-14, xvi, 535).
	2.84 second determination.	3.37 Van Slyke by group method (Van Slyke, D.D., <i>J. Biol. Chem.</i> , 1913-14, xvi, 537).
		2.59 (Abderhalden, E., <i>Z. physiol. Chem.</i> , 1905, xlii, 23)
Edestin.	3.04	2.40 (Osborne, T. B., and Liddle, L. M., <i>Am. J. Physiol.</i> , 1910, xxvi, 295.)

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*Hemoglobins.*

	Colorimetric.	Other methods.
	<i>per cent</i>	<i>per cent</i>
Horse (1)	8.9	10.5 (Abderhalden, E., <i>Z. physiol. Chem.</i> , 1902-03, xxxvii, 492.)
(2)	8.65	
Cat.	8.5	
	8.55	
Sheep.	8.8	
Ox.	7.93	
		8.15 (Van Slyke, D. D., <i>J. Biol. Chem.</i> 1911-12, x, 52.)

Whole blood (normal human).

1.5731 gm. of histidine per 100 cc. of hydrolyzed blood.

Blood serum (normal human).

0.2338 gm. of histidine per 100 cc. of hydrolyzed blood serum.

## EXPERIMENTAL.

*Sodium Phosphotungstate Does Not Interfere with the Color Produced When Histidine Reacts with p-Phenyldiazonium Sulfonate.*

Phosphotungstic acid—15 gm.—was dissolved in 85 cc. of water contained in a 100 cc. volumetric flask. Histidine dichloride solution—2 cc. of a 1 per cent solution—was then added together with enough water to give a total volume of 100 cc. The liquid remained clear. Of this solution, 5 cc. were transferred to a 25 cc. volumetric flask, neutralized with a sodium hydroxide solution, and diluted to 25 cc. Of this diluted solution

0.50 cc. had a color value equivalent to 10.0 mm. (CR-MO)<sup>10</sup>

1.00 " " " " " " " 19.5 " (CR-MO)

For the 0.50 cc. portion this is equal to 0.02 gm. of histidine dichloride for the entire original liquid, 100 per cent of the amount actually introduced.

For the 1.00 cc. portion this is equal to 0.0195 gm. of histidine dichloride for the entire original liquid, 97.5 per cent of the amount actually introduced.

<sup>10</sup> The details of the colorimetric method and an explanation of the symbols employed here have been described.<sup>8</sup> This paper also contains tables by means of which colorimetric readings can be readily transformed into gm. of histidine, histamine, etc.

*These results show that sodium phosphotungstate, in concentrations as high as 15 per cent, does not interfere with the accuracy of the colorimetric determination of histidine.*

*The Solubility of Histidine Phosphotungstate.*

*Experiment A.*—Histidine dichloride—0.1000 gm.—was mixed with 82 cc. of water and 18 cc. of 37 per cent hydrochloric acid in a 300 cc. Pyrex flask. The solution was then treated with 100 cc. of a 30 per cent aqueous solution of phosphotungstic acid. The clear liquid was placed in the ice chest. At the end of 24 hours a precipitate of histidine phosphotungstate had formed and collected on the bottom of the flask. To ascertain how much histidine remained in solution 1 cc. of the clear supernatant liquid was transferred to a 10 cc. precision cylinder, neutralized to litmus paper with sodium hydroxide, and diluted to 10 cc. Of this solution

0.50 cc.	had a color value equivalent to	7.0 mm.	(CR-MO)
1.00 " " " "	" " " "	14.1 " "	(CR-MO)

This, by table,<sup>10</sup> shows that the equivalent of 0.056 gm. of histidine dichloride was still in solution in 200 cc. of liquid at the end of 24 hours.

The precipitate was triturated with a glass rod, the mixture then being allowed to stand in the ice chest for 43 more hours. At the end of this time 1 cc. of the clear supernatant liquid was removed, neutralized, and diluted to 5 cc. Of this solution

0.50 cc.	had a color value equivalent to	2.5 mm.	(CR-MO)
1.00 " " " "	" " " "	4.8 " "	(CR-MO)

This, by table, shows that the 200 cc. of precipitation liquid still contained the equivalent of 0.010 gm. of histidine dichloride which is equal to 0.0068 gm. of histidine base. Longer standing or cooling to 0° in an ice bath produced no change in this value.

To ascertain the effect of rise in temperature upon the solubility of histidine phosphotungstate, the mixture was allowed to stand on the laboratory table for 24 hours at a room temperature that varied from 27–32°. Then 1 cc. of the clear supernatant



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liquid was removed, neutralized, and diluted to 5 cc. as before. Of this solution

0.50 cc. had a color value equivalent to 13.3 mm. (CR-MO)

This, by table, is equivalent to 0.0532 gm. of histidine dichloride—0.0364 gm. of histidine base—for the entire 200 cc. of precipitation liquid.

*From this experiment it is clear that histidine phosphotungstate precipitates slowly and that its solubility is markedly influenced by changes in temperature.*

*Experiment B.*—A precipitation was now conducted under the conditions specified by Van Slyke.<sup>7</sup> Histidinedi chloride—0.1000 gm.—was mixed with 82 cc. of water and 18 cc. of 37 per cent hydrochloric acid in a 300 cc. flask. The solution was then treated with 100 cc. of a 15 per cent aqueous solution of phosphotungstic acid. After standing for 2 hours in the ice chest, crystals had begun to form. They were disintegrated with a glass rod. This gave rise to a rapid and copious precipitation of histidine phosphotungstate. After standing for 24 hours in the ice chest, 1 cc. of the clear supernatant liquid was removed, neutralized, and diluted to 5 cc. Of this solution

0.50 cc. had a color value equivalent to 3.1 mm. (CR-MO)

1.00 " " " " " " " 6.1 " (CR-MO)

This, by table, is equivalent to 0.012 gm. of histidine dichloride per 200 cc. of precipitation liquid. Longer standing in the ice chest produced no change in this value.

The flask was now transferred to an ice bath, where it was kept for 24 hours. At the end of this time, 1 cc. of the clear supernatant liquid was removed, neutralized, and diluted to 5 cc. Of this solution

0.50 cc. had a color value equivalent to 2.1 mm. (CR-MO)

1.00 " " " " " " " 4.2 " (CR-MO)

This, by table, shows that the equivalent of 0.0084 gm. of histidine dichloride—0.00571 gm. of histidine base—was left in solution in the 200 cc. of precipitation liquid.

This experiment shows that, to obtain a most complete and uniform precipitation of histidine phosphotungstate under the

conditions specified by Van Slyke, it is necessary to cool the precipitation mixture in an ice bath. The precipitation, although it is slightly more complete when the concentration of phosphotungstic acid is 7.5 per cent than it is when the concentration is 15 per cent, is not very markedly influenced by the concentration of phosphotungstic acid in the liquid.

*The Effect of Other Amino-Acids upon the Colorimetric Determination of Histidine.*<sup>11</sup>

*Effect of Cystine.*

*Standard Cystine Solution.*—Chemically pure cystine—1.0000 gm.—was dissolved in 30 cc. of N HCl and the resulting solution diluted to exactly 100 cc.

The test solutions were prepared by mixing 1 cc. of a 1 per cent histidine dichloride solution with 2 to 20 cc. of the standard cystine solution and diluting the resulting liquid to 100 cc. The colorimetric determinations were then carried out on 0.20 and 0.40 cc. portions of this test solution.

Table I shows that when cystine and histidine are present in the proportion of 3 (cystine) to 1 (histidine), which is the proportion that is encountered in the cystine-rich keratins, the colorimetric determination of histidine is not interfered with to the slightest extent. The first indication of an interference is obtained when the ratio of cystine to histidine is 6 to 1—a proportion that has not been encountered heretofore in any protein—and under these conditions the interference is only about 3 per cent. A serious interference occurs only when the ratio of cystine to histidine assumes the extremely artificial ratio of 29 (cystine) to 1 (histidine).

From these results we conclude that cystine will not interfere with the colorimetric determination of histidine in any of the known proteins.

<sup>11</sup> We wish to call attention to the fact that the interference percentages given in the following pages are correct only when chemicals of highest purity are used in compounding the alkaline reagent. For the past year we have been using Baker and Adamson's highest purity anhydrous sodium carbonate and sodium nitrite (crystals of 97 to 100 per cent purity) and J. T. Baker's sulfanilic acid.

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That the unsaturated sulfur atoms in cystine are responsible for the interference noted for large quantities of this substance is rendered highly probable by the fact that a solution containing equal parts by weight of sodium sulfide and histidine gives rise to a yellowish brown color with *p*-phenyldiazonium sulfonate that is only 40 per cent as intense as that obtained with a pure histidine solution.

TABLE I.  
*The Effect of Cystine on the Colorimetric Determination of Histidine.*

Concentration of histidine per 100 cc.	Concentration of cystine per 100 cc.	Test solution used for the determination.	Theoretical color value.	Color value as determined.	Interference.
<i>mg.</i>	<i>mg.</i>	<i>cc.</i>	<i>mm.</i>	<i>mm.</i>	<i>per cent</i>
6.8	20	0.20	10.0	10.0	None.
		0.40	20.0	20.0	
6.8	30	0.20	10.0	10.0	None.
		0.40	20.0	20.0	
6.8	40	0.20	10.0	9.7	3
		0.40	20.0	19.5	
6.8	50	0.20	10.0	9.6	4
		0.40	20.0	19.3	
6.8	100	0.20	10.0	9.2	8
		0.40	20.0	18.1	
6.8	200	0.20	10.0	7.6	24
		0.40	20.0	14.7 Color distinct; too yellow.	

*Effect of Leucine.*

*Standard Leucine Solution.*—Chemically pure leucine—2.0000 gm.—was dissolved in 20 cc. of N HCl and the resulting solution diluted to exactly 200 cc.



The test solutions were prepared by mixing 1 cc. of a 1 per cent histidine dichloride solution with 10 to 80 cc. of the standard leucine solution and diluting the resulting liquid to 100 cc. The colorimetric determinations were then carried out on 0.20 and 0.40 cc. portions of the test solution.

Table II shows that leucine does not interfere with the colorimetric determination of histidine until the ratio of leucine to histidine has been raised to the extremely exaggerated proportion of 114 (leucine) to 1 (histidine). While one would not, of course, expect the presence of leucine together with the diamino-acids in

TABLE II.

*The Effect of Leucine on the Colorimetric Determination of Histidine.*

Concentration of histidine per 100 cc.	Concentration of leucine per 100 cc.	Test solution used for the determination.	Theoretical color value (CR-MO).	Color value as determined.	Interference.
mg.	mg.	cc.	mm.	mm.	per cent
6.8	100	0.20	10.0	10.0	None.
		0.40	20.0	20.0	
6.8	500	0.20	10.0	10.0	None.
		0.40	20.0	20.0	
6.8	800	0.20	10.0	9.6	4
		0.40	20.0	19.4 Color slightly too yellow.	

the phosphotungstic acid precipitate, we believed that it might be desirable to show that the presence of a typical  $\alpha$ -amino-acid does not interfere with the quantity and intensity of color produced by histidine.

#### *Effect of Arginine.*

*Standard Arginine Solution.*—A solution containing arginine was prepared from salmon sperm by hydrolyzing with sulfuric acid and precipitating the arginine first with silver nitrate and baryta and then with phosphotungstic acid. The solution of

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arginine sulfate obtained by decomposing the phosphotungstate with a cold solution of baryta and removing the barium with a slight excess of sulfuric acid had the following properties.

1. 2 cc. gave 1.70 cc. of amino  $N_2$  at  $28^\circ$  and 752 mm. The solution must, therefore, have contained 0.571 gm. of arginine per 100 cc.

2. Of the above solution, 20 cc. were treated with 0.18 gm. of picrolonic acid dissolved in 3 cc. of alcohol. The picrolonate obtained after drying for 10 hours at  $110^\circ$  weighed 0.2893 gm. and decomposed at  $233^\circ$ .

3. The solution contained no ammonia.

4. When 10 cc. of the solution were mixed with 15 cc. of water and 12.5 gm. of KOH and hydrolyzed for 6 hours as specified by

TABLE III.

*The Effect of Arginine on the Colorimetric Determination of Histidine.*

Concentration of histidine per 100 cc.	Concentration of arginine per 100 cc.	Test solution used for determination.	Theoretical color value.	Color value as determined.	Interference.
mg.	mg.	cc.	mm.	mm.	per cent
6.8	115	0.20	10.0	10.0	None.
		0.40	20.0	20.0	
6.8	230	0.20	10.0	9.7	3
		0.40	20.0	19.1	

Van Slyke,<sup>7</sup> sufficient ammonia was evolved to neutralize 6.8 cc. of 0.1 N HCl. The ammonia evolved from this quantity of a 0.571 per cent solution of arginine should have neutralized 6.56 cc. of the 0.1 N acid.

The test solutions were prepared by mixing 1 cc. of a 1 per cent histidine dichloride solution with 20 and 40 cc. of the standard arginine solution and diluting the resulting liquid to 100 cc. The colorimetric determinations were then carried out on 0.20 and 0.40 cc. portions of the test solution.

Table III shows that arginine does not interfere with the colorimetric determination of histidine until the ratio of arginine to histidine has been raised to the proportion of 34 (arginine) to 1 (histidine). Since a ratio of 6 (arginine) to 1 (histidine) is

the largest that has been encountered in any protein heretofore, we conclude that arginine will not interfere with the colorimetric determination of histidine in any of the known proteins.

*The Colorimetric Estimation of Histidine in Proteins.*

*Casein.*

Duplicate experiments were carried out on 3 gm. of carefully purified vacuum-dried casein by the method outlined in the introduction. The volume of the test liquid was 500 cc. Of this solution

0.10 cc. had a color value equivalent to 11.7 mm. (CR-MO)
0.20 " " " " " " " 23.4 " (CR-MO)

The color was exactly like that produced by histidine. *The duplicates checked exactly.* This, by table, is equivalent to 0.0000234 gm. of histidine dichloride per 0.10 cc. or 0.117 gm. per entire 500 cc. of test liquid. This is equal to 0.0796 gm. of histidine base in 3 gm. of casein if no correction is made for the solubility of histidine phosphotungstate. We have found that 0.00571 gm. of histidine remains in solution in 200 cc. of precipitation liquid under these conditions; *therefore casein—3 gm.—contains 0.08531 gm. of histidine which is 2.84 per cent.*

*Edestin.*

Weight dried at 110°, gm.....	3.0000
Precipitation volume, cc.....	200.0
Volume of the test liquid, cc.....	1,000.0
Colorimetric readings:	
0.10 cc. had a color value equivalent to	6.3 mm.
0.20 " " " " " " " "	12.6 " (CR-MO)
0.30 " " " " " " " "	18.9 "
Histidine dichloride by table, gm.....	0.126
" base in test liquid, gm.....	0.0856
Solubility correction blank, gm.....	0.00571
Histidine in edestin sample, gm.....	0.09131
" " " " per cent.....	3.04



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### *Horse Hemoglobin.*<sup>12</sup>

*Sample 1.*—Horse hemoglobin—1.9000 gm.—that had been dried *in vacuo* over sulfuric acid for 48 hours, was analyzed for histidine by the method outlined in the introduction. The volume of the pale yellow test liquid was 1,000 cc. Of this solution

0.10 cc. had a color value equivalent to 12.0 mm. (CR-MO)  
0.20 " " " " " " " 24.0 " (CR-MO)

The color was exactly like that produced by histidine. This, by table, is equivalent to 0.24 gm. of histidine dichloride—0.1632 gm. of histidine base—for the entire 1,000 cc. of test liquid. This value must be raised by 0.00571 gm.; the solubility correction blank; *therefore horse hemoglobin—1.9000 gm.—contains 0.16891 gm. of histidine which is equal to 8.9 per cent.*

*Sample 2.*<sup>13</sup>—The air-dried material—2.0000 gm.—was dried *in vacuo* over sulfuric acid for 48 hours. The solid so obtained—1.8664 gm.—was heated to constant weight at 110°. The final product—1.8430 gm.—was then analyzed for histidine by the previously described method. The volume of the test liquid was 1,000 cc. of which

0.10 cc. had a color value equivalent to 11.3 mm. (CR-MO)  
0.20 " " " " " " " 22.5 " (CR-MO)

This, by table, is equivalent to 0.226 gm. of histidine dichloride—0.1536 gm. of histidine base—for the entire 1,000 cc. of test liquid. To this must then be added 0.00571 gm., the solubility correction blank; *therefore this sample of horse hemoglobin—1.8430 gm.—contained 0.1593 gm. of histidine which is equal to 8.65 per cent.*

### *Cat Hemoglobin.*

Duplicate analyses were carried out on the same sample.

<sup>12</sup> The four varieties of hemoglobin were kindly furnished by W. H. Welker, Professor of physiological chemistry, University of Illinois, Medical Department.

<sup>13</sup> The two samples of hemoglobin differed both in color and in physical structure.

	<i>First Analysis.</i>	<i>Second Analysis.</i>
Weight air-dried, gm.....	2.0000	
“ vacuum-dried, gm.....	1.8742	
“ dried at 110°, gm.....	1.8561	1.1142
Precipitation volume, cc.....	200.00	100.0
Volume of the test liquid, cc.....	1,000.00	1,000.0
Colorimetric readings:		
0.10 cc. had a color value equivalent to.....	11.2 mm.	6.8 mm.
0.20 cc. had a color value equivalent to.....	22.4 “	13.6 “
Histidine dichloride by table, gm.....	0.224	0.136
“ base in test liquid, gm.....	0.1522	0.0925
Solubility correction blank, gm.....	0.00571	0.00285
Histidine in hemoglobin sample, gm...	0.1579	0.09535
Histidine in hemoglobin sample, <i>per cent.</i> .....	8.50	8.55

*Sheep Hemoglobin.*

Weight vacuum-dried, gm.....	2.0000
Precipitation volume, cc.....	200.0
Volume of test liquid, cc.....	1,000.0
Colorimetric readings:	
0.10 cc. had a color value equivalent to..	12.5 mm. (CR-MO)
0.20 “ “ “ “ “ “ “ “	25.0 “ (CR-MO)
Histidine dichloride by table, gm.....	0.250
“ base in test liquid, gm.....	0.170
Solubility correction blank, gm.....	0.00571
Histidine in hemoglobin sample, gm.....	0.17571
“ “ “ “ “ <i>per cent.</i> ...	8.8

*Ox Hemoglobin.*<sup>14</sup>

Weight air-dried, gm.....	1.3640
“ vacuum-dried, gm.....	1.2690
“ dried at 110°, gm.....	1.2542
Precipitation volume, cc.....	150.0
Volume of test liquid, cc.....	1,000.0
Colorimetric readings:	
0.10 cc. had a color value equivalent to..	7.0 mm. (CR-MO)
0.20 “ “ “ “ “ “ “ “	14.0 “ (CR-MO)
Histidine dichloride by table, gm.....	0.140
“ base in test liquid, gm.....	0.0952
Solubility correction blank, gm.....	0.00428
Histidine in hemoglobin sample, gm.....	0.09948
“ “ “ “ “ <i>per cent.</i> ...	7.93

<sup>14</sup> This sample charred slightly while it was being hydrolyzed. The percentage of histidine may, therefore, be somewhat low.

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### *Whole Blood (Normal Human).*

Whole blood—exactly 10 cc.—to which a few crystals of ammonium oxalate had been added to prevent coagulation, was mixed with 15 cc. of 37 per cent hydrochloric acid and hydrolyzed in the customary manner. The resulting liquid was then analyzed for histidine by using the method outlined in the introduction.

Precipitation volume, cc.....	200.0
Volume of test liquid, cc.....	1,000.0
Colorimetric readings:	
0.10 cc. had a color value equivalent to..	11.1 mm. (CR-MO)
0.20 “ “ “ “ “ “ “ “ ..	22.2 “ (CR-MO)
Histidine dichloride by table, gm.....	0.222
“ base in test liquid, gm.....	0.1516
Solubility correction blank, gm.....	0.00571
Histidine in 10 cc. of blood, gm.....	0.15731
“ “ 100 “ “ “ gm.....	1.5731

### *Blood Serum (Normal Human).*

Whole blood—about 30 cc.—was drawn into a centrifuge tube and allowed to clot slowly in the ice chest. The mixture was centrifuged to free the serum as completely as possible from fibrin and blood cells. The perfectly clear, pale yellow serum—exactly 10 cc.—was withdrawn by means of a pipette, mixed with 15 cc. of 37 per cent HCl, and hydrolyzed in the customary manner. The resulting liquid was then analyzed for histidine by using the method outlined in the introduction.

Precipitation volume, cc.....	200.0
Volume of test liquid, cc.....	500.0
Colorimetric readings:	
0.25 cc. had a color value equivalent to...	6.5 mm. (CR-MO)
0.50 “ “ “ “ “ “ “ “ ...	13.0 “ (CR-MO)
Histidine dichloride by table, gm.....	0.0260
“ base in test liquid, gm.....	0.01767
Solubility correction blank, gm.....	0.00571
Histidine in 10 cc. of serum, gm.....	0.02338
“ “ 100 “ “ “ gm.....	0.2338



## STUDIES ON PROTEINOGENOUS AMINES.

### VIII. A METHOD FOR THE QUANTITATIVE COLORIMETRIC ESTIMATION OF HISTAMINE IN PROTEIN AND PROTEIN-CONTAINING MATTER.

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In a series of articles published recently<sup>1</sup> we communicated a method by means of which small quantities of histamine,  $\beta$ -imidazolethylamine, can be accurately determined in a simple

<sup>1</sup> Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 497, 521, 539.

synthetic culture medium. We stated at that time, that the method as it was employed in the bacterial metabolism studies was not applicable, in its entirety, to more complex mixtures such as blood and urine. The present communication contains the description of a purely chemical method by means of which the histamine content of tissues and other protein-containing matter can be accurately determined.

### *I. Description of the Method.*

1. *Preliminary Treatment of the Material.*—A dry solid was usually hydrolyzed immediately without preliminary treatment. A hydrated tissue, like blood, liver, or hypophysis, was mixed with sufficient alcohol to give a final alcohol concentration of 75 per cent. A few drops of acetic acid were then added and the resulting mixture was heated on the water bath for 1 to 2 hours, to extract the free histamine and to coagulate the proteins. The mixture was then cooled and filtered on a Buchner funnel, the residue being washed with 95 per cent alcohol. This divides the material into two fractions, the *alcoholic extract A* and the *alcohol-insoluble residue R*. Each fraction is then freed from alcohol by heating on the water bath after which it is ready to hydrolyze.

2. *Hydrolysis.*—The dry solid is mixed with ten to twenty parts of 20 per cent hydrochloric acid and hydrolyzed by boiling, under a reflux condenser, for 30 hours over an electrically heated sand bath.

3. *Removal of the Hydrochloric Acid.*—The hydrochloric acid is removed by distillation *in vacuo* at 60° from the same flask. The residue is finally dried *in vacuo* at 80° for 1 hour.

4. *Removal of Ammonia.*—The residue is dissolved in ten to twenty parts of water and the solution treated with an excess of lime and a volume of 95 per cent alcohol equal to one-half the volume of the water added. The mixture is then subjected to a distillation *in vacuo* at 40° until its volume has been reduced to about one-half. This removes the ammonia completely.

5. *Removal of Humin.* The mixture is filtered, on a Buchner funnel, from humin and excess lime, the precipitate being carefully

washed with a large excess of hot water until the washings give a negative Pauly reaction.

6. *Preparation of the Phosphotungstates.*—The alkaline filtrate is acidified by adding a slight excess of hydrochloric acid. The resulting clear liquid is then evaporated to dryness on the water bath in a glass dish. Because of the variability in the quantity and quality of the original protein-containing matter, a concise statement cannot be made as to the amount of phosphotungstic acid to employ. In general, 4 gm. of phosphotungstic acid are sufficient to precipitate the hexone bases from 1 gm. of dry protein. A quantity of phosphotungstic acid equal to twice the weight of the total solids of a hydrated tissue is usually sufficient to precipitate completely the hexone bases. In all cases the concentration of the hydrochloric acid should be 9 cc. of the 37 per cent acid per 100 cc. of precipitation liquid. The final volume of the precipitation liquid should not exceed 2,000 cc. The phosphotungstates are prepared at water bath temperatures and the mixture is then allowed to cool slowly to room temperature after which it is cooled in an ice bath for 24 hours and filtered with suction. The precipitate is washed with an ice-cold fluid containing 18 cc. of 37 per cent hydrochloric acid and 15 gm. of phosphotungstic acid per total aqueous volume of 200 cc.

7. *Decomposition of the Phosphotungstates.*—The phosphotungstate precipitate which contains the histamine, together with histidine, arginine, lysine, cystine, tyramine, and possibly other amines, is suspended in a large volume of hot water—500 to 4,000 cc.—and treated with an excess of a hot saturated solution of baryta. The resulting mixture is digested for 1 hour on the water bath, after which it is cooled in tap water and filtered on a Buchner funnel, the precipitate being thoroughly washed with hot water. The filtrate is heated on the water bath and freed from excess barium by the careful addition of  $N H_2SO_4$ . The mixture is filtered hot through a folded filter. The filtrate, which should contain a trace of barium, is then evaporated to dryness in a glass dish. The residue is dissolved in the smallest possible quantity of water, the solution transferred to a graduated cylinder, and made up to the smallest convenient volume—10 to 100 cc. (Liquid 7).



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8. *Extraction of Histamine with Amyl Alcohol. First Colorimetric Determination.*—10 cc. of Liquid 7 are transferred to a glass-stoppered, shake out bottle,<sup>2</sup> mixed with 3 gm. of solid sodium hydroxide, and extracted six times with redistilled<sup>3</sup> amyl alcohol using 20 cc. for each extraction. The combined amyl alcohol extracts are then extracted five times with N H<sub>2</sub>SO<sub>4</sub> using 20 cc. for the first and 10 cc. for each of the remaining four extracts. This process is repeated until all Liquid 7 has been extracted.

The combined acid extracts are heated on the water bath and exactly neutralized with baryta. The hot mixture is filtered from barium sulfate, and the filtrate evaporated to dryness in a small glass dish. This residue contains all the histamine. Because of the concentrated character of Liquid 7, a very small quantity of the amino-acids also passes into the amyl alcohol as sodium salts. A colorimetric determination is always positive at this point, because of the presence of histidine. To remove the histidine entirely, the residue obtained above is transferred to the shake out bottle with 10 cc. of water, the solution treated with 3 gm. of solid sodium hydroxide, and extracted with amyl alcohol as above. The sulfuric acid extracts finally obtained are neutralized exactly with baryta. The mixture is filtered from barium sulfate and the filtrate, which should contain no barium, is evaporated to dryness in a small glass dish. The pale yellow residue is dissolved in water and diluted to 25 or 50 cc. Histamine is then estimated colorimetrically in this fraction using the method previously described by us.<sup>1</sup> If the colorimetric test indicates the presence of histamine, the remaining liquid is treated according to Section 9 below. If there is the slightest reason to believe that some of the histidine has again been extracted by the amyl alcohol, which will be apparent from the speed of the color development,<sup>1</sup> a third extraction with amyl alcohol should be carried out. When these extractions are properly conducted the histamine always passes quantitatively into the amyl alcohol.

<sup>2</sup> For a detailed description of this extraction process see Koessler and Hanke,<sup>1</sup> pp. 525-529.

<sup>3</sup> It is best to distill the amyl alcohol *in vacuo*.

9. *Precipitation with Silver Nitrate and Baryta. Second Colorimetric Determination.*—The liquid obtained under Section 8 is diluted to 100 cc. in a 300 cc. Pyrex flask and mixed with 5 cc. of a 20 per cent silver nitrate solution. To the clear liquid is then added barium hydroxide—8 gm.—dissolved in 50 cc. of warm water. The resulting dark brown mixture is filtered with suction on a platinum cone. The precipitate is washed with 50 cc. of a cold saturated solution of baryta. The filtrate should be clear. This divides the material into two fractions, the silver precipitate, which contains the histamine, and the silver filtrate, which although it can contain only traces of histamine seems almost invariably to contain substances that are physiologically active.

The silver precipitate is suspended, with the filter paper, in 50 cc. of water and mixed with 3 cc. of 37 per cent HCl and enough of a 20 per cent  $\text{Na}_2\text{SO}_4$  solution to remove the barium completely. The mixture is digested on the water bath for 1 hour after which it is filtered and the precipitate washed with hot water. The clear colorless filtrate is neutralized exactly with sodium hydroxide and evaporated to a small volume in a glass dish. The colorless to pale yellow liquid is diluted with water to 25 or 50 cc. Histamine is then estimated colorimetrically in this fraction. If the test shows the presence of histamine, this can be verified biologically on 5 cc. of the liquid and chemically on 20 cc. of the liquid according to Section 10.

This precipitation with silver nitrate and baryta is necessary for two reasons. Test Liquid 8 occasionally contains substances that interfere with the color reaction to such an extent that an accurate determination of histamine is impossible. Such interfering substances appear to be of two kinds; namely, those that *inhibit* the production of color by histamine without giving a color themselves, and those that impart a yellow or green color to the liquid which, of course, makes a perfect match impossible. *These interfering substances were always found to remain in the silver filtrate;* the histamine appears quantitatively in the silver precipitate.

Although one is accustomed to think of histidine, histamine, tyrosine, and tyramine as the only substances that give a positive Pauly reaction, certain of the body tissues appear to contain

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small quantities of other substances that give a very similar color reaction. These substances are at least partially removed by the precipitation with silver nitrate and baryta.

10. *Extraction of Histamine by Means of Chloroform and Methyl Alcohol. Third Colorimetric Determination.*—The liquid obtained under Section 9—20 cc.—is evaporated to dryness *in vacuo* in a 500 cc. long necked, round bottomed flask. The perfectly dry residue is treated with 10 cc. of chemically pure methyl alcohol and 0.50 gm. of potassium hydroxide. The alkaline liquid is then treated with 200 cc. of redistilled chloroform and placed in the ice chest for 15 hours. The mixture is filtered through a small folded filter. The precipitate is washed with 200 cc. of hot chloroform. The chloroform extracts are mixed with a few drops of 37 per cent HCl and subjected to a vacuum distillation to remove the chloroform and the methyl alcohol. The residue is dissolved in water and redistilled *in vacuo* to remove the methyl alcohol completely. The residue finally obtained is dissolved in water, diluted to 20 cc., and examined colorimetrically for histamine.

A final purification of the histamine by means of chloroform in doubtful cases seemed desirable because of the fact that chloroform, although it dissolves histamine, will not dissolve many substances that are soluble in amyl alcohol. We tried first to extract histamine from the dry residue obtained by evaporating a histamine solution—1 cc. of a 1 per cent solution—with 2 gm. of sodium carbonate. The quantity of histamine that passed into the chloroform varied from 60 to 80 per cent of the starting material when the extractions were carried on for 24 hours in a Soxhlet extractor under apparently identical conditions.

When lime was used in place of sodium carbonate, only 27 per cent of the histamine passed into the chloroform.

We tried then to extract histamine from an alkaline aqueous solution by means of chloroform. This proved to be impractical because histamine is too soluble in water and too little soluble in chloroform. Such an extraction would be almost endless.

The following principles are incorporated in the successful process that was described in detail above. Methyl alcohol is a good solvent for histamine, salts of histamine, and potassium



hydroxide. When methyl alcohol is used in conjunction with potassium hydroxide, the *histamine is brought into solution as free base*; so there is no possibility of its being occluded by chloroform-insoluble substances. The addition of chloroform to the methyl alcohol solution of histamine precipitates inorganic salts and most of the potassium hydroxide but the histamine remains in solution.

*II. Proof that Histamine can be Quantitatively Recovered by the Methods Described in Sections 6, 7, 9, and 10 of Part I.*

1. *Histamine Quantitatively Precipitated by Phosphotungstic Acid.*—Phosphotungstic acid—37 gm.—and 37 per cent hydrochloric acid—45 cc.—were dissolved in water and the solution was diluted to 500 cc. Histamine dichloride<sup>4</sup> solution—0.50 cc. of a 1 per cent solution—was then added to the above liquid. The resulting mixture was cooled in an ice bath for 2 hours and filtered with suction. The precipitate was suspended in 200 cc. of hot water. The remaining steps were carried out as described in Section 7, Part I. The residue finally obtained was dissolved in water and the solution diluted to 100 cc. Of this solution

0.20 cc. had a color value equivalent to 7.5 mm. (CR-MO)

0.40 “ “ “ “ “ “ “ 15.0 “ (CR-MO)

which, by table, is equivalent to 0.0050 gm. of histamine dichloride, 100 per cent of the amount introduced.

*From this experiment it would seem that histamine phosphotungstate is practically insoluble in the precipitation liquid employed at a temperature of 0°.* This conclusion was verified by performing a qualitative Pauly reaction on 10 cc. of the filtrate from the histamine phosphotungstate. The reaction was entirely negative.

2. *Histamine Quantitatively Precipitated by Silver Nitrate and Baryta.*—The liquid obtained above—100 cc.—which still contained 5 mg. of histamine dichloride, was precipitated with silver nitrate and baryta as described in Section 9, Part I. The liquid

<sup>4</sup> See Koessler, K. K., and Hanke, M. T., *J. Am. Chem. Soc.*, 1918, xl, 1716, for a description of the method used in preparing the histamine dichloride.

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finally obtained after removing the silver and barium from the silver *precipitate*, was diluted to 100 cc. Of this solution

0.20 cc. had a color value equivalent to 7.5 mm. (CR-MO)  
0.40 " " " " " " " 15.0 " (CR-MO)

which, by table, is equivalent to 0.0050 gm. of histamine dichloride for the entire test liquid, 100 per cent of the amount originally introduced.

*This experiment proves that histamine is quantitatively precipitated by silver nitrate and baryta under the conditions specified above.*

3. *Extraction of Histamine by Means of Chloroform and Methyl Alcohol.*—Histamine dichloride solution—1.00 cc. of a 1 per cent solution—was evaporated to dryness *in vacuo* in a 500 cc. long necked, round bottomed flask. The dry residue was then treated as described in Section 10, Part I. The residue finally obtained by distillation of the chloroform extracts was dissolved in water and diluted to 100 cc. Of this solution

0.10 cc. had a color value equivalent to 7.2 mm. (CR-MO)  
0.20 " " " " " " " 14.4 " (CR-MO)

which, by table, is equivalent to 0.0096 gm. of histamine dichloride, 96 per cent of the amount originally introduced.

### III. *The Method Applied to Casein with and without the Addition of Histamine.*

The search for histamine in a pure protein would seem superfluous if it were not for the fact that Abel and Kubota<sup>5</sup> have recently claimed to have found it, or a physiologically and chemically similar homologue, as a constituent of casein, egg albumin, and edestin. We were not entirely convinced that the physiologically active substance obtained by the above authors was histamine, because they did not identify their substance as histamine chemically and because the presence of this amine as a normal constituent of a pure protein, that has not been allowed to putrefy, seemed rather improbable. Since casein of high purity is easily

<sup>5</sup> Abel, J. J., and Kubota, S., *J. Pharmacol. and Exp. Therap.*, 1919, xiii, 243.

prepared, we decided to use this protein as one check on the accuracy of our method.

*Analysis of Casein for Histamine.*

Carefully purified casein—40 gm.—prepared from fresh skimmed milk, was mixed with 800 cc. of 20 per cent hydrochloric acid and hydrolyzed by boiling for 30 hours.

The hydrochloric acid, ammonia, and humin were removed as described in Sections 3, 4, and 5, Part I.

The phosphotungstates were precipitated from a total volume of 2,000 cc. with 150 gm. of phosphotungstic acid. The precipitation liquid contained 180 cc. of 37 per cent HCl.

The phosphotungstate precipitate was suspended in 4,000 cc. of hot water and freed from phosphotungstic acid, excess barium, etc., as described in Section 7, Part I. The final volume of Liquid 7 was 20 cc.

The double amyl alcohol extraction of Liquid 7 was carried out as described in Section 8, Part I. The final volume of the test solution was 200 cc., of which 0.20 cc. had no color value and 1.00 cc. gave a pale green color that was quite unlike that of an imidazole and which resembled that produced by ammonia and the aliphatic amines. An accurate comparison with the standard indicator solution was, of course, impossible. By comparing intensities, the green color was found to have an intensity value equivalent to about 3.0 mm. (CR-MO).

There can be little doubt that this sample of casein contained no histamine. If the above *green* color is, nevertheless, ascribed to histamine, 0.0008 gm. of this amine, calculated as hydrochloride, is the maximum amount that could have been present in 40 gm. of casein.

The remainder of the test liquid was evaporated to dryness in a small glass dish. The residue was dissolved in 2 cc. of water and the solution injected into a cat that had been anesthetized and prepared so that a blood pressure and respiratory tracing could be obtained at the same time.<sup>6</sup> The tracing obtained,

<sup>6</sup> In obtaining the blood pressure tracings in this and in the subsequent work we enjoyed the aid of our colleague, Dr. Julian H. Lewis, which we herewith thankfully acknowledge.



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Fig. 1, shows that the injection produced a drop in blood pressure entirely similar to that produced by histamine. Some substance or substances are present that behave very similarly to histamine

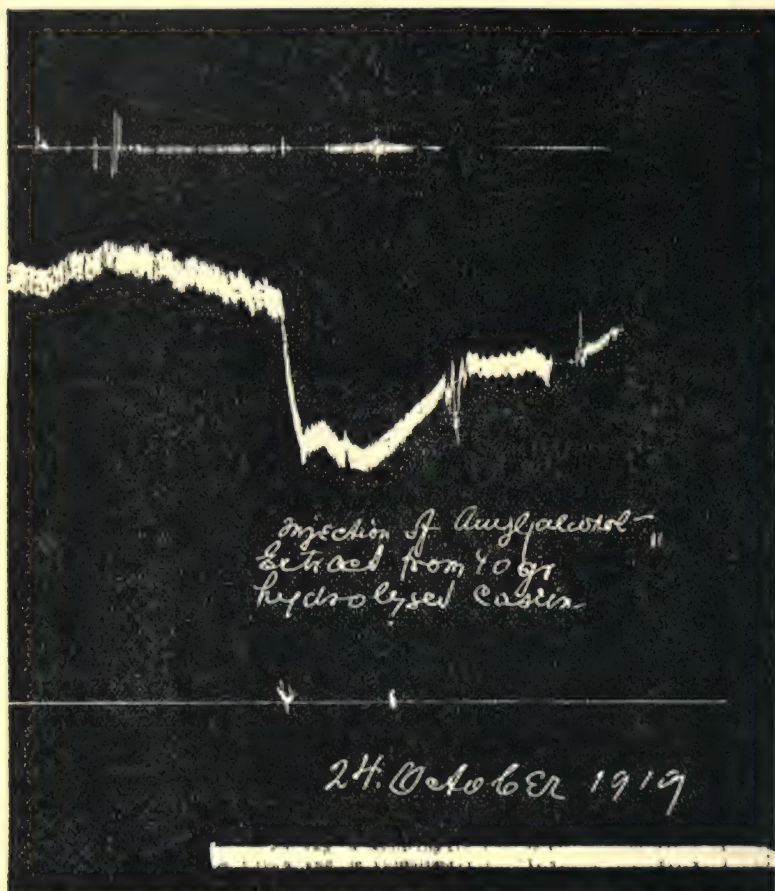


FIG. 1. Blood pressure tracing obtained by injecting the entire amyl alcohol extract and 40 gm. of casein into the femoral vein of a cat.

pharmacologically. We have, therefore, been able to verify the pharmacological findings of Abel and Kubota as illustrated in Fig. 16 of their article; but we cannot conclude with them that casein contains histamine.

*Histamine That Has Been Added to Casein Can Be Recovered Quantitatively.*

To be certain that our method would recover a small quantity of histamine and that our conclusions concerning casein were correct, another experiment was conducted on 40 gm. of casein exactly like the one that has just been described excepting that 0.01 gm. of histamine dichloride was mixed with the casein before hydrolysis. The final volume of the test liquid was 100 cc., of which

0.05 cc.	had a color value equivalent to	4.0 mm.	(CR-MO)
0.10 " " " "	" " " "	8.0 "	(CR-MO)
0.20 " " " "	" " " "	15.2 "	(CR-MO)

The color for the 0.05 and 0.10 cc. portions was exactly like that produced by histamine. The color obtained with the 0.20 cc. portion was slightly yellow, and, as can be seen from the above value, there was a slight interference with the color production in this case. Since the values obtained with the two smaller portions checked exactly, the calculations were based upon the 0.10 cc. portion. The reading obtained is equivalent to 0.0107 gm. of histamine dichloride for the entire test liquid which is 107 per cent of the amount actually introduced. Hydrolyzed casein alone gave a green color, the intensity value of which was equivalent to 0.0008 gm. of histamine dichloride. This quantity must, therefore, be subtracted from the above gross value to obtain the amount of histamine dichloride actually recovered which is 0.0099 gm. or 99 per cent of the amount actually introduced.

*From these two experiments we conclude that the method gives reliable results and that casein contains no histamine although a substance pharmacologically similar to histamine can be split from casein by acid hydrolysis.*

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### IV. *The Method Applied to Human Blood Serum after the Addition of Histamine.*

Human blood serum<sup>7</sup>—75 cc.—was mixed with 0.50 cc. of a 1 per cent solution of histamine dichloride. The resulting solution was then treated with 225 cc. of 95 per cent alcohol and a few drops of glacial acetic acid. The mixture was digested for 1 hour on the water bath, cooled to room temperature, and filtered with suction, the coagulum being washed with 95 per cent alcohol. This divided the material into two fractions, *the alcoholic extract A* and *the alcohol-insoluble residue R*.

#### *Alcoholic Extract A.*

The alcoholic extract was freed from water and alcohol by distillation *in vacuo* at 50°. The dry residue was treated with 100 cc. of 20 per cent HCl and hydrolyzed as usual. *The material charred considerably* during the process of hydrolysis.

The hydrochloric acid, ammonia, and humin were removed as described in Part I. The phosphotungstates were precipitated from a total volume of 200 cc. with 15 gm. of phosphotungstic acid. The phosphotungstate precipitate was suspended in 800 cc. of hot water and freed from phosphotungstic acid with baryta. The final volume of Liquid 7 was 10 cc. The double amyl alcohol extraction of Liquid 7 was carried out as described in Part I. The final volume of the test solution was 50 cc., of which

0.10 cc. had a color value equivalent to 5.2 mm. (CR-MO)  
0.20 " " " " " " " 9.5 " (CR-MO)

The color was exactly like that produced by histamine; but the two readings did not check exactly. A precipitation with silver nitrate and baryta was, therefore, conducted to remove the interfering substances.

The precipitation with AgNO<sub>3</sub> and baryta was carried out as described in Section 9, Part I.

The final volume of the test liquid was 50 cc., of which

0.10 cc. had a color value equivalent to 5.2 mm. (CR-MO)  
0.20 " " " " " " " 10.3 " (CR-MO)

---

<sup>7</sup> A sample of this serum was analyzed for histamine by the direct process described on p. 544. The serum contained no histamine.



This, by table, is equivalent to 0.0035 gm. of histamine dichloride.

*Alcohol-Insoluble Residue R.*

The dried material was mixed with 200 cc. of 20 per cent HCl and hydrolyzed as usual.

The hydrochloric acid, ammonia, and humin were removed as described in Part I.

The phosphotungstates were precipitated from a total volume of 400 cc. with 40 gm. of phosphotungstic acid. The precipitate was suspended in 2,000 cc. of water and freed from phosphotungstic acid and baryta. The final volume of Liquid 7 was 20 cc.

The double amyl alcohol extraction of Liquid 7 was carried out as described in Part I. The final volume of the test solution was 50 cc. of which 1.0 cc. had an intensity value of 4.2 mm. The color was far too yellow for histamine. A further purification of this fraction was effected by means of a silver precipitation.

The precipitation with  $\text{AgNO}_3$  and baryta was carried out as described in Section 9, Part I. The final volume of the test liquid was 50 cc., of which

0.50 cc.	had a color value equivalent to 2.1 mm.	(CR-MO)
1.00 " " " " "	" " " " " " " 4.2 "	(CR-MO)

The color was too yellow for histamine but the time of development was correct for this imidazole. This, by table, is equivalent to 0.0003 gm. of histamine  $\text{Cl}_2$  for the entire test liquid.

In this experiment, 3.5 mg. of histamine dichloride appeared in the alcoholic extract and 0.3 mg. appeared in the alcohol-insoluble residue. The total recovery was 3.8 mg. Since 5 mg. were originally introduced, 1.2 mg. of histamine dichloride were lost somewhere in the process. We have previously shown<sup>1</sup> that histamine is readily adsorbed by charcoal. The alcoholic extract A charred considerably during the process of hydrolysis; so it is possible that the 1.2 mg. of histamine  $\text{Cl}_2$  were adsorbed by the charcoal formed at this time. To prove that this statement is correct, the following experiment was carried out in which the possibility of charcoal formation was entirely eliminated.

The same sample of blood serum 75 cc. was mixed with 0.50 cc. of a 1 per cent solution of histamine dichloride. The resulting solution was treated with 225 cc. of 95 per cent alcohol and a few drops of glacial acetic acid. The mixture was digested

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for 1 hour on the water bath, cooled to room temperature, and filtered with suction, the coagulum being washed with 95 per cent alcohol. The alcoholic extract A was evaporated to dryness in a small glass dish. The residue was transferred to the shake out bottle with 10 cc. of water and the solution treated with 3 gm. of solid NaOH.

A double amyl alcohol extraction was then carried out<sup>8</sup> as described in Section 8, Part I. The final volume of the test liquid was 100 cc., of which

0.20 cc.	had a color value equivalent to	7.2 mm.	(CR-MO)
0.40 " " " " " "	" " " " " "	" 14.4 "	(CR-MO)

The color was exactly like that produced by histamine. This, by table, is equivalent to 0.0048 gm. of histamine dichloride. Since the alcohol insoluble residue was previously proved to adsorb the equivalent of 0.0003 gm. of histamine dichloride, the total recovery in this case was 0.0051 gm. as against 0.0050 gm. actually introduced.

This leaves little doubt that the 1.2 mg. of histamine dichloride that were lost in the hydrolysis experiment had been adsorbed by the charcoal that was formed during the process of hydrolysis.

### SUMMARY.

1. A colorimetric method is described by means of which quantities of histamine ( $\beta$ -imidazolyethylamine) as small as 0.1 mg. can be accurately determined in protein and protein-containing matter.

2. The presence of histamine could not be demonstrated in 40 gm. of casein by this method.

3. Casein contains a depressor substance that is similar to histamine pharmacologically.

4. Histamine, that has been added to casein before the latter is hydrolyzed, can be recovered quantitatively.

5. The presence of histamine could not be demonstrated in 75 cc. of human blood serum by this method.

6. Histamine that has been added to the blood serum before the latter is hydrolyzed can be recovered quantitatively.

<sup>8</sup> We wish to call attention to the fact that in this case Steps 2 to 8 of the general process described in Part I have been eliminated. Although this process is very simple and rapid, it can be used only to estimate loosely combined histamine. Peptamine histamine would probably not pass into the amyl alcohol.







## STUDIES ON PROTEINOGENOUS AMINES.

### IX. IS HISTAMINE A NORMAL CONSTITUENT OF THE HYPOPHYSIS CEREBRI?

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(Received for publication, June 21, 1920.)

#### INTRODUCTION.

The physiological activity of extracts of the hypophysis has been a subject of constant investigation since Oliver and Schäfer discovered in 1895 that such extracts had the power of raising the blood pressure of animals on intravenous injection. Howell, 3 years later, brought experimental proof that it was only the posterior part of the hypophysis which possessed this pressor action. This elevation of pressure seemed to result from the direct action of the active substance upon the involuntary muscle of the heart and arteries without the intervention of the sympathetic nervous system. This specific affinity of the active principle of the hypophysis for the smooth muscle fiber cell was confirmed anew through the important discovery of Dale that pituitary extracts have the faculty of producing intense contractions of the uterus. The detection of this oxytocic action led to the introduction of pituitary extracts into human therapeutics. Since then, the isolation of the active principle of the hypophysis has been repeatedly attempted by biochemists.<sup>1</sup> Although our knowledge has been greatly increased by these investigations, the actual chemical composition of the active principle of the hypophysis is still unknown.

<sup>1</sup> For a review of the work and literature on the chemistry of the pituitary gland see Crawford, A. C., *J. Pharmacol. and Exp. Therap.*, 1920, xv, 81.

There are two facts that seem to stand out from the sum total of these investigations. The active principle of the gland behaves like an amine, and it seems always to be associated with an imidazole derivative. These two chemical properties claimed for the active principle of the hypophysis, in conjunction with the physiological activity of the extracts on the uterus, suggested a connection with  $\beta$ -imidazolyethylamine (histamine). Since this amine is derived from histidine by decarboxylation, Barger suggested that "The pituitary active principle is possibly a polypeptid like derivative of histidine." Decarboxylated polypeptides of this type have been synthesized by Guggenheim who proposed for them the name peptamines. They show the same physiological behavior as the amine from which they are derived; but to a much less degree.

This whole question seemed to be definitely settled in a very simple manner when Abel and Kubota<sup>2</sup> published a paper in which they state that "histamine is the plain muscle-stimulating and depressor constituent of the posterior lobe of the pituitary gland." This statement is based upon the actual isolation and identification of 18 mg. of histamine dipicrate from 1 pound of dried substance of the whole pituitary gland. It is difficult to accept the conclusions of these authors, because of the differences in chemical as well as physiological behavior of pituitary extracts and histamine.

There is a widespread conception that histamine is a very labile substance. Thus, for example, Myers and Voegtlin<sup>3</sup> suggested recently in connection with some work on the chemical isolation of vitamins that the physiological activity of a crystalline product containing histamine or histamine-like substances was destroyed by drying. This certainly does not hold true for histamine; for this amine loses none of its physiological activity when it is dried at room temperature or at a temperature of 100° in air or *in vacuo*. We have shown in a previous publication<sup>4</sup> that histamine, when it is heated on the boiling water bath with concentrated sodium hydroxide for 7 hours, is only destroyed to

<sup>2</sup> Abel, J. J., and Kubota, S., *J. Pharmacol. and Exp. Therap.*, 1919, **xiii**, 243.

<sup>3</sup> Myers, C. N., and Voegtlin, C., *Proc. Nat. Acad. Sc.*, 1920, **vi**, 3.

<sup>4</sup> Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, **xxxix**, 539.



the extent of 7.5 per cent, while 10 hours of boiling with hot concentrated hydrochloric acid leaves the histamine unchanged. Guggenheim<sup>5</sup> showed in 1914 that the pressor and oxytocic properties of "pituglandol" are completely destroyed even by dilute alkali—2.0 N NaOH—at room temperature, while the physiological activity of histamine on bronchi and blood pressure was not in the least impaired by the same treatment. The differences in physiological activity of the extracts of the hypophysis and of histamine, with especial regard to their broncho-constrictor action, have been admirably discussed by Jackson and Mills.<sup>6</sup>

Experiments on cases of diabetes insipidus in man have shown that the reaction of the pituitary extract in suppressing the polyuria for days cannot be duplicated by injections of histamine (Rowntree).

The typical urticaria-like wheal produced on application of histamine to the scarified skin of man cannot be obtained with pituitary extracts.<sup>7</sup>

We have developed quantitative methods, in our laboratory, for the estimation of histamine and other imidazoles in tissues and were applying these methods to a detailed study of the presence of histamine in the animal organism at the time of the appearance of the article by Abel and Kubota. Considering the far reaching importance of the hypophysis problem it seemed logical to extend our research to the hypophysis. From the results of this part of our work we have to conclude that *the perfectly fresh hypophysis contains no histamine*. Still there can be no question that Abel and Kubota isolated histamine from the pound of pituitary substance which they secured in a dry state on the market. The question is, how did the histamine get into the pituitary material used by these investigators? It is a well known fact<sup>4</sup> that histamine is readily formed from histidine by the action of certain putrefactive microorganisms; and histidine is a normal constituent of practically all protein of animal origin. The method used at the slaughter houses for

<sup>5</sup> Guggenheim, M., *Biochem. Z.*, 1914, lxx, 189.

<sup>6</sup> Jackson, D. E., and Mills, C. A., *J. Lab. and Clin. Med.*, 1919, v, 1.

<sup>7</sup> Sollmann, T., and Pilcher, J. D., *J. Pharmacol. and Exp. Therap.*, 1916-17, ix, 309.

preparing dried glandular products gives ample opportunity for changes, both autolytic and bacterial. Our work on the relation of histamine to peptone shock (page 567) emphasizes again how essential it is to control bacterial action and how imperative it is to work only with material that has been prepared by the investigators themselves.

We have been able to demonstrate the presence of histamine in the liver and feces of one dog by the method used in the present investigation and we expect to report on this phase of the work in the near future; but we were unable to demonstrate the presence of the slightest trace of histamine in the hypophysis. *On the basis of this work, the claim of Abel and Kubota to have found in histamine the plain muscle stimulating and depressor constituent of the posterior lobe of the pituitary gland seems to us untenable.*

#### EXPERIMENTAL.

##### *Collection of the Material.*

The perfectly fresh glands—beef—were collected at Swift and Company's Chicago plant. They were trimmed free from extraneous tissue, weighed, and dropped into boiling absolute alcohol approximately 25 minutes after the animal had been struck. The total weight of the moist glands was 346 gm.

The alcohol was removed by distillation *in vacuo* at 40°. The residue was put through a food chopper. The finely divided material was then digested for 2 hours with 2,000 cc. of 75 per cent alcohol to which a few cc. of glacial acetic acid had been added. The mixture was cooled and filtered with suction, the residue being washed thoroughly with 95 per cent alcohol. This divided the material into two fractions, the alcoholic extract A and the alcohol-insoluble residue R.

##### *The Alcoholic Extract A.*

The alcohol and water were removed by distillation *in vacuo* at 50°. The residue was emulsified with hot water and diluted to 140 cc. in a graduated cylinder. This fraction might contain histamine either free or in the form of a peptamine. Since it seemed desirable to know not only how much histamine was

present but also the form in which it was present in the gland, this fraction was divided into two equal parts. One-half was extracted directly with amyl alcohol without hydrolysis, the other half was hydrolyzed, treated with phosphotungstic acid, and then extracted with amyl alcohol.

*Loosely Bound Histamine. Non-Hydrolyzed Alcoholic Extract A.*—This fraction which was not hydrolyzed, was extracted with amyl alcohol, 10 cc. at a time, as described in Part I of the preceding article, page 544. The combined amyl alcohol extracts were then extracted with  $N H_2SO_4$ ,<sup>8</sup> the acid extracts neutralized with baryta, the mixture was filtered from  $BaSO_4$ , and the filtrate evaporated to dryness in a glass dish. The residue was transferred to a shake out bottle with 10 cc. of water. Sodium hydroxide—3 gm.—was added and the liquid extracted six times with 20 cc. of amyl alcohol. The combined amyl alcohol extracts were extracted with  $N H_2SO_4$ , using 20 cc. for the first and 10 cc. for each of the remaining four extracts. The acid extracts were neutralized with baryta, the mixture was filtered from  $BaSO_4$ , and the filtrate, which was free from barium, was evaporated to dryness in a small glass dish. The residue was dissolved in water, the solution transferred to a 50 cc. graduated precision cylinder, and diluted with water to 50 cc. Of this solution

0.20 cc. had a color value equivalent to 3.1 mm.  $(CH_3-MO)$   
 0.50 " " " " " " " 7.5 "  $(CH_3-MO)$

The color resembled that produced by histamine excepting in its time of development which was too rapid for histamine. This, by table, is equivalent to 0.004 gm. of histamine dichloride for the entire test liquid.

The remaining liquid was subjected to a phosphotungstic acid precipitation as described in Sections 6, 7, and 8, Part I, of the preceding paper. A colorimetric determination showed that all the color-producing substance had been precipitated by the phosphotungstic acid.

<sup>8</sup> The details of this process are given in Section 8, Part I, of the preceding article.



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The liquid was then subjected to a precipitation with silver nitrate and baryta as described in Section 9, Part I, of the preceding paper.

The fraction *precipitated by silver* was finally brought to a volume of 50 cc. Of this solution

0.50 cc. had no perceptible color value.  
1.00 cc. gave a very faint green color.

This would indicate that the color-producing substance that was soluble in amyl alcohol and precipitated by phosphotungstic acid *could not have been histamine* because it did not give an *insoluble silver compound*.

The silver filtrate, which contained the color-producing substance, did not contract the excised virgin guinea pig uterus.

We conclude, therefore, that the perfectly fresh hypophysis contains no loosely bound or free histamine.

*Peptamine Histamine. Hydrolyzed Alcoholic Extract A.*—This fraction—70 cc., one-half of the entire alcoholic extract A—was mixed with 70 cc. of 37 per cent HCl and hydrolyzed by boiling for 30 hours over an electrically heated sand bath. The material was freed from HCl, NH<sub>3</sub>, and humin, precipitated with phosphotungstic acid,<sup>9</sup> and extracted with amyl alcohol as described in Sections 1 to 9, Part I, of the preceding paper.

The final volume of test Liquid 8 was 100 cc., of which

0.50 cc. had a color value equivalent to 2.7 mm. (CR-MO)  
1.00 " " " " " " " 5.5 " (CR-MO)

The color resembled that produced by histamine excepting in its time of development which was too rapid for histamine. This, by table, is equivalent to 0.00074 gm. of histamine dichloride for the entire test liquid.

The remainder of the above solution was subjected to a precipitation with silver nitrate and baryta as described in Section 9, Part I, of the preceding paper. The fraction *precipitated by silver* was finally brought to a volume of 50 cc., of which

0.50 cc. had a color value equivalent to 2.0 mm. (CR-MO)  
1.00 " " " " " " " 3.8 " (CR-MO)

---

<sup>9</sup> The volume of the precipitation liquid was 250 cc. 20 gm. of phosphotungstic acid were employed.

This, by table, is equivalent to 0.00025 gm. of histamine dichloride for the entire test liquid.

The remaining liquid was evaporated to dryness. The residue was dissolved in 5 cc. of water. *Of this solution, 1 cc. did not contract the excised virgin guinea pig uterus.* This proves that the alcoholic extract from the hypophysis contained no histamine. The slight color value of this fraction, which we found to be equivalent to 0.00025 gm. of histamine dichloride, must therefore have been due to some physiologically inert substance. *It could not have been due to histamine.*

#### *Recapitulation.*

The 75 per cent alcoholic extract from 346 gm. of fresh hypophysis does not contain histamine.

#### *The Alcohol-Insoluble Residue R.*

The dry residue, which weighed 132 gm., was mixed with 1,500 cc. of 20 per cent hydrochloric acid and hydrolyzed in the customary manner.

The material was freed from HCl, NH<sub>3</sub>, and humin, and precipitated with phosphotungstic acid<sup>10</sup> as described in Sections 1 to 8, Part I, of the preceding paper. The residue finally obtained from the phosphotungstate *precipitate*, was nearly solid and large in volume. Fully 300 cc. of water were required to bring this residue into solution. This would have required so many amyl alcohol extractions that a new procedure was adopted. The solution was treated with 20 gm. of anhydrous sodium carbonate. The resulting liquid was evaporated on the water bath and the residue dried for 48 hours *in vacuo* over sulfuric acid. The perfectly dry, brown solid was then extracted with chloroform for 72 hours in a Soxhlet extractor. This divided the material into two fractions, the *chloroform extract*, which should contain *most* of the histamine, and the *chloroform-insoluble residue*.

<sup>10</sup> The volume of the precipitation liquid was 2,000 cc. 250 gm. of phosphotungstic acid were employed.

*The Chloroform Extract.*

The combined chloroform extracts were evaporated on the water bath. The residue was dissolved in water and diluted to 50 cc. Of this solution 1.00 cc. gave a faint *green* color that could most certainly not have been produced by histamine. The solution was evaporated to dryness. The residue was dissolved in water and diluted to 5 cc. *Of this solution, 1.00 cc. did not contract the excised virgin guinea pig uterus.*

This proves that the chloroform extract, which should certainly have contained *some* histamine if any had been present in the original solid, was entirely free from histamine.

*The Chloroform-Insoluble Residue.*

To be perfectly certain that the histamine had not been adsorbed by the solids in this residue so that none of it was extracted by the chloroform, the solid was extracted three times with hot 95 per cent alcohol, using 200 cc. for each extraction. The combined alcoholic extracts were evaporated on the water bath. The residue was dissolved in water, the solution treated with sodium hydroxide, and the alkaline liquid extracted as usual with amyl alcohol. The test liquid finally obtained had a volume of 50 cc. of which

0.20 cc.	had an intensity value equivalent to 5.0 mm.	(CR-MO)
1.00 " " "	" " " " " " " " 8.0 "	(CR-MO)

As can be readily seen, the above values are worthless because the 1 cc. portion gave almost the same color as the 0.20 cc. portion. To eliminate the interfering substances, a silver precipitation was now conducted as described in Section 9, Part I, of the preceding paper. The final volume of the test liquid was 25 cc., of which

0.10 cc.	had a color value equivalent to 8.2 mm.	(CR-MO)
0.20 " " " " "	" " " " " " " " 16.2 "	(CR-MO)

The color was brown, attained its full intensity almost immediately, and was perfectly stable so that it did not resemble the color produced by histamine in any particular. If, nevertheless,



the color value is calculated as histamine dichloride, the presence of 0.00275 gm. of this substance is indicated.

That the above color value was not due to histamine was proved as follows:

1. *1 cc. of the test liquid did not contract the excised virgin guinea pig uterus.*

2. That the substance responsible for the color production was different from histamine chemically was proved by subjecting the remainder of the liquid—23 cc.—to the chloroform methyl alcohol purification described in Section 10, Part I, of the preceding paper. The residue finally obtained was dissolved in water and diluted to 23 cc. Of this solution

0.50 cc. had a color value equivalent to 8.6 mm. (CR-MO)

1.00 " " " " " " 17.0 " (CR-MO)

The color was like that previously obtained and not like that produced by histamine. By table, this would be equivalent to 0.00057 gm. of histamine dichloride for the entire test liquid, which is the maximum amount of this amine that could have been present in the entire 346 gm. of moist glands originally employed. That histamine could not have been responsible for even this slight color value was previously proved by the fact that the liquid after the silver precipitation did not contract the excised virgin guinea pig uterus.

#### CONCLUSION.

Perfectly fresh beef hypophysis does not contain histamine.









## STUDIES ON PROTEINOGENOUS AMINES.

### X. THE RELATION OF HISTAMINE TO PEPTONE SHOCK.

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(Received for publication, June 21, 1920.)

#### INTRODUCTION.

The discovery that the intravenous injection of proteose-peptone into animals produces profound systemic disturbances of shock-like character was made by Schmidt-Muhlheim<sup>1</sup> and Fano<sup>2</sup> in the laboratory of Ludwig. Ludwig and his pupils were studying the mode of absorption of proteins from the intestinal tract, their fate, and their resynthesis in the animal organism. In an endeavor to settle the question whether proteins are mainly absorbed as proteoses or peptones or as amino-acids, proteose-peptone solutions were injected into the circulation of animals (dogs). While the primary inquiry was not definitely answered by these experiments, they resulted in the observation that the animals injected showed a very marked fall of blood pressure, acceleration of lymph flow, incoagulability of the blood, sub-normal temperature, increased frequency of respiration, and a state of loss of consciousness (peptone shock).

In interpreting this toxic action of the proteose-peptones two distinct views have crystallized from the work of a large number of investigators.<sup>3</sup> One group believes that the toxicity of the proteose-peptone is a property inherent in the molecular struc-

<sup>1</sup> Schmidt-Muhlheim, A., *Arch. Physiol.*, 1880, 33.

<sup>2</sup> Fano, *Arch. Physiol.*, 1881, 277.

<sup>3</sup> For the complete literature of the subject see Chittenden, R. H., Mendel, L. B., and Henderson, Y., *Am. J. Physiol.*, 1898-99, ii, 142; and especially Underhill, F. P., and Hendrix, B. M., *J. Biol. Chem.*, 1915, xxii, 443.

ture of these products of protein digestion. The other group ascribes their toxic action to the presence of contaminating substances which can be extracted from the proteose-peptone mixture; the latter thus freed from the supposedly true poison is said to have no physiological activity. Brieger's peptotoxin,<sup>4</sup> Pick and Spiro's peptozyme,<sup>5</sup> and Popielski's vasodilatin<sup>6</sup> are substances of undetermined chemical composition that are supposed to represent the poison which can be separated from the peptone. Though the work of Nolf, Underhill, Zunz, and Gibson seemed to have proved definitely that proteose-peptones of vegetable as well as of animal origin are toxic in themselves, the doctrine of a separable toxin in peptone has been recently revived by Abel and Kubota,<sup>7</sup> who state that *histamine is the toxic agent of Witte's peptone*. A possible relation of  $\beta$ -imidazolyethylamine to peptone shock had been previously suggested by Dale and Laidlaw,<sup>8</sup> who emphasized the great similarity in the symptoms of peptone shock to those produced by the injection of histamine. The whole symptom-complex of peptone intoxication was revived and the older work on this subject brought again to the attention of the biologists through the studies of Biedl and Kraus<sup>9</sup> on anaphylaxis. They recalled first the striking resemblance of the symptoms produced in animals sensitized to a certain protein on reinjection of this protein with the syndrome obtained by Ludwig's pupils on peptone injection. In a later publication they consider anaphylaxis as a true peptone intoxication. Dale and Laidlaw never regarded the correspondence of symptoms as a sufficient basis for theoretical speculations. That they are still of the same opinion can be seen from the following statement in their paper on histamine shock.<sup>10</sup>

<sup>4</sup> Brieger, L., *Z. physiol. Chem.*, 1882-83, vii, 274. For a criticism of Brieger's peptotoxin work, see Salkowski, E., *Virchows Arch. path. Anat.*, 1891, cxxiv, 409.

<sup>5</sup> Pick, E. P., and Spiro, K., *Z. physiol. Chem.*, 1900-01, xxxi, 235.

<sup>6</sup> Popielski, L., *Arch. ges. Physiol.*, 1909, cxxvi, 483.

<sup>7</sup> Abel, J. J., and Kubota, S., *J. Pharmacol. and Exp. Therap.*, 1919, xiii, 243.

<sup>8</sup> Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1910-11, xli, 318.

<sup>9</sup> Biedl, A., and Kraus, R., *Wien. klin. Woch.*, 1909, xxii, 363. Kraus, R., and Levaditi, C., *Handbuch der Technik und Methodik der Immunitätsforschung*, Jena, 1911, i, 255-290.

<sup>10</sup> Dale, H. H., and Laidlaw, P. P., *J. Physiol.*, 1919, lii, 355.



"The existence of these points of community, in the action of substances so utterly unrelated chemically as histamine and certain metallic ions, forbids any assumption that the production of similar effects, by unknown constituents of some organ or tissue, indicates the presence therein of histamine itself, or of any substance chemically related to it. The similarity depends on the fact that all act on the endothelium, and produce in it changes probably of the same general type. A hint, as to what the nature of these changes may be, is possibly provided by the fact that the anaphylactic reaction, more especially in those species in which histamine exhibits this type of action, also presents the picture of an acute endothelial poisoning. All available evidence goes to show that the anaphylactic antibody is of the nature of a "precipitin," the interaction of which with the corresponding antigen results in a change in the state of dispersion of the colloidal particles."

Abel and Kubota, though well acquainted with Dale and Laidlaw's paper, take the view that histamine is present wherever living protoplasm exists, or at least wherever protoplasm is killed. Believing that it makes its appearance wherever a true protein is disrupted by enzymes, acids, or other hydrolytic agents, they believe they have demonstrated its presence in ereptone, Witte's peptone, casein, and edestin. Traumatic shock, anaphylaxis, Vaughan's protein poison, the active principle of the posterior part of the hypophysis, and many other questions of fundamental importance to medicine they believe are settled on the basis of the presence and physiological activity of histamine. To bring about the solution of all these questions in such a simple manner is so suggestive and attractive that it is only with a certain sense of reluctance that we venture the opinion that the far reaching conclusions of Abel and Kubota are not justified by the data of their experimental work. In only one case have they actually demonstrated the presence of histamine; namely, in a dried commercial sample of hypophysis glands.

We have discussed the relation of histamine to the hypophysis problem in the preceding paper. In working on the presence of histamine in protein products Abel and Kubota used two commercial preparations, ereptone and Witte's peptone. Physiological tests made with solutions prepared from an impure picrate led them to believe that the shock poison of ereptone and Witte's peptone is histamine. The results obtained with these commercial preparations are directly transferred to peptone

shock in general. Bacterial putrefaction was not excluded in the preparation of the protein derivatives used; thus the fundamental question, so far reaching for this whole problem and recognized as such by the above authors in the introduction to their paper,<sup>11</sup> namely if histamine can be formed in the absence of bacteria by the enzymatic activity of organ cells alone, is still unanswered.

Vaughan's poisonous protein fraction is not identical with histamine, for its toxicity is completely destroyed by boiling with dilute (3 per cent) hydrochloric acid,<sup>12</sup> while heating histamine with concentrated hydrochloric acid for 10 hours on the boiling water bath leaves the imidazole compound intact,<sup>13</sup> and does not impair its toxicity.

To expect a similarity in physiological action from compounds having a similar chemical structure is logical and justified by evidence; but to conclude from a similar pharmacodynamic action to the identity of chemical structure or the invariable presence of one and the same substance is a perilous undertaking.

#### *Plan of Procedure.*

The main postulates of an endeavor to clear up the relation of histamine to peptone shock seemed to be:

1. The preparation of a proteose-peptone under sterile conditions. Since certain microorganisms have the faculty of forming histamine from protein matter, no conclusions can be drawn regarding the origin of histamine found in a sample of peptone if this postulate is not fulfilled. We controlled the absence of bacteria during the time of the preparation of our peptone by daily preparations of aerobic and anaerobic cultures.

2. The peptone must be physiologically active. That this was actually the case can be seen by consulting Figs. 1 and 2.

<sup>11</sup> Abel and Kubota,<sup>7</sup> pp. 247-249.

<sup>12</sup> Underhill, F. P., and Hendrix, B. M., *J. Biol. Chem.*, 1915, xxii, 465.

<sup>13</sup> Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 521.

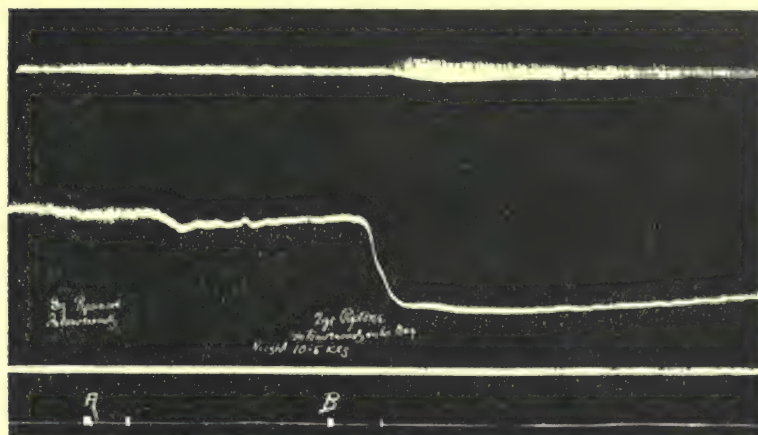


FIG. 1. (A) Injection of pepsin. (B) Injection of 2 gm. of histamine-free peptone prepared from fibrin.

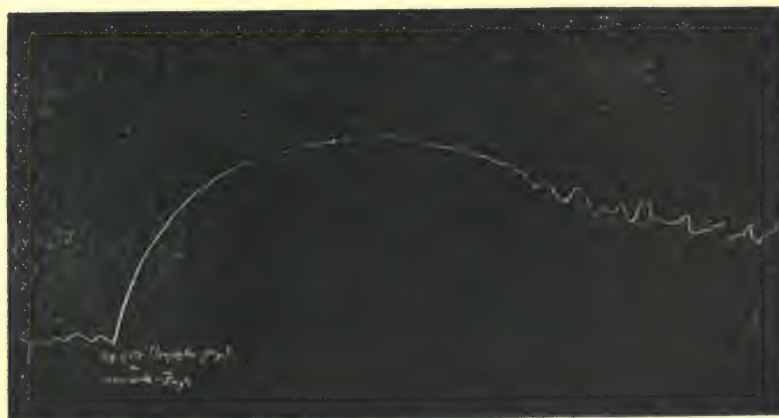


FIG. 2. Contraction of the excised virgin guinea pig uterus produced by 0.1 gm. of a histamine-free, fibrin peptone. The uterus was suspended in 100 cc. of Locke-Ringer solution.



## EXPERIMENTAL.

*Preparation of Histamine-Free Peptone from Fibrin.*<sup>14</sup>

Well washed blood fibrin—275 gm.—was treated with 11,000 cc. of 0.2 per cent sodium hydroxide. The mixture was covered with a layer of toluene and allowed to stand at room temperature for 8 days. The opalescent mixture was filtered through several layers of toweling. The filtrate—10,000 cc.—was diluted with 10,000 cc. of water. Acetic acid—0.5 per cent—was then added in small portions until a perfect flocculation had occurred. The precipitate was washed four times, by decantation, with distilled water and was then largely freed from water by filtration on a Buchner funnel. The residue obtained—116 gm.—was mixed with 350 cc. of 0.4 per cent hydrochloric acid and 30 cc. of a 0.1 per cent pepsin solution in 0.4 per cent hydrochloric acid. The mixture was covered with toluene and incubated at 37° for 1 week. That the liquid was free from living micro-organisms throughout this entire period was proved by repeated aerobic and anaerobic cultures. The resulting mixture was nearly neutralized with a 10 per cent sodium carbonate solution, heated to boiling, and filtered through a large, water-soaked, folded filter paper. The filtrate was evaporated to dryness *in vacuo* over sulfuric acid, at room temperature. The dry, pale yellow powder weighed 14.5 gm. This will be referred to as the “fibrin peptone.”

*Analysis of Fibrin Peptone for Histamine.*

5 gm. of the peptone were dissolved in 100 cc. of 20 per cent hydrochloric acid and hydrolyzed by boiling for 30 hours over an electrically heated sand bath. The acid, ammonia, and humin were removed as previously described.<sup>15</sup> The phosphotungstates were precipitated from a total volume of 400 cc. with 40 gm. of

<sup>14</sup> We are indebted to Dr. F. C. Koch, Department of Physiological Chemistry, University of Chicago, for the details of the method used in the preparation of the peptone.

<sup>15</sup> A detailed description of the method used in the estimation of histamine in protein-containing matter has been reported (Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1920, xliii, 543).

phosphotungstic acid. The phosphotungstate precipitate was freed from phosphotungstic acid, etc. The residue finally obtained was subjected to a double amyl alcohol extraction. The volume of the test liquid was 50 cc. of which, tested by our meth-

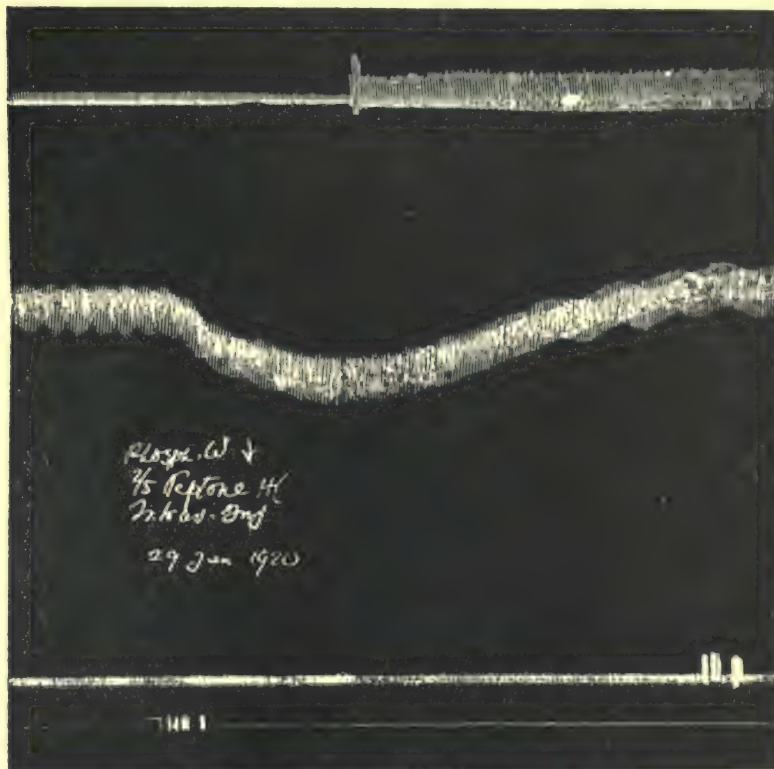


FIG. 3. The peptone was hydrolyzed, precipitated with phosphotungstic acid, and the phosphotungstate precipitate extracted twice with amyl alcohol from an alkaline solution. The liquid was free from histamine. The 2 cc. injected represent the amyl alcohol extract from 2 gm. of peptone.

ods, 0.50 cc. gave no perceptible color and 1.00 cc. gave a very faint green color. *This proves that the above peptone was entirely free from histamine.*

The remaining liquid was evaporated on the water bath. The dry residue was dissolved in 5 cc. of water. Of the resulting solution, 1.00 cc. did not contract the excised virgin guinea pig uterus; which substantiates the conclusion previously arrived at that histamine was absent. Of the same solution, 2 cc. were injected into the femoral vein of a dog. Fig. 3 shows that this injection produced a slight drop in blood pressure which must, therefore, have been produced by some substance not identical with histamine.

*A Typical Peptone Shock from a Histamine-Free Peptone.*—2 gm. of the above peptone, that had been proved to contain no histamine, were dissolved in 6 cc. of isotonic salt solution and injected into the femoral vein of a dog that had been anesthetized and arranged so that blood pressure and respiratory tracings could be obtained. As can be seen by examining Fig. 1, this injection gave rise to a typical peptone shock. A morphological blood examination showed before the injection of the peptone, hemoglobin 88, leucocytes 20,600, erythrocytes 6,250,000; 1 hour after the injection, hemoglobin 108, leucocytes 5,700, and erythrocytes 7,200,000.

*The Action of Peptone on the Unstriated Muscle of the Guinea Pig Uterus.*—That Witte's peptone has the property of contracting the isolated uterus of the virgin guinea pig in a manner that is indistinguishable from that of histamine has been shown by Dale and Laidlaw.<sup>8</sup> Abel and Kubota, who worked with a chloroform extract from Witte's peptone, ascribe this oxytocic reaction of peptone to histamine. That this action is also inherent in the structure of the peptone molecule and not dependent upon the presence of histamine, we showed by using our fibrin peptone, which had been proved to be free from histamine.

5 cc. of a 2 per cent solution of fibrin peptone—0.1 gm. of peptone—were introduced into 100 cc. of Locke-Ringer solution in which an excised guinea pig uterus was suspended. A typical contraction of the uterus was obtained which is illustrated by the curve of Fig. 2.

To prove that the reactions were produced by the peptone obtained from fibrin and not by some constituent of the pepsin employed in the preparation of the peptone, 30 cc. of a 0.1 per cent pepsin solution in 0.4 per cent HCl were incubated for



1 week, neutralized with sodium carbonate, evaporated to dryness, dissolved in 2 cc. of water, and injected into the femoral vein of a dog. An examination of Fig. 1, Part A, shows that this injection did not produce a fall in blood pressure.

*Analysis of Witte's Peptone for Histamine.*—Witte's peptone—5 gm.—was hydrolyzed, freed from hydrochloric acid, ammonia, and humin, precipitated with phosphotungstic acid, and extracted with amyl alcohol, the details being identical with those just described for the fibrin peptone. The volume of test Liquid 8 was 25 cc., of which

0.50 cc. had an intensity value equivalent to 2.6 mm. (CR-MO)  
1.00 “ “ “ “ “ “ “ 4.1 “ (CR-MO)

The color was orange-yellow and hence difficult to match against the standard indicator solution. The remaining liquid—23.5 cc.—was subjected to a precipitation with silver nitrate and baryta.<sup>15</sup> The volume of test Liquid 9 was 23.5 cc., of which

0.50 cc. had a color value equivalent to 2.5 mm. (CR-MO)  
1.00 “ “ “ “ “ “ “ 5.0 “ (CR-MO)

The color was somewhat more yellow than that produced by histamine; but the time of development was correct for this imidazole. This, by table, is equivalent to 0.0001675 gm. of histamine dichloride for the entire test liquid.

The remaining liquid—22 cc.—was evaporated on the water bath. The residue was dissolved in 5 cc. of isotonic salt solution. Of this solution, 1.00 cc. contracted the excised virgin guinea pig uterus. The curve obtained, Fig. 4, was exactly like that obtained with a solution of pure histamine dichloride.

This sample of Witte's peptone contained the equivalent of 0.00335 gm. of histamine dichloride per 100 gm. of peptone.

*Histamine that Has Been Added to Peptone Can Be Recovered Quantitatively.*—This experiment was a duplicate of the one that has just been described excepting that 0.0050 gm. of histamine dichloride was added to the 5 gm. of Witte's peptone before hydrolysis. The final volume of test Liquid 8 was 100 cc., of which

0.20 cc. had a color value equivalent to 7.7 mm. (CR-MO)  
0.40 “ “ “ “ “ “ “ 15.4 “ (CR-MO)

The color was exactly like that produced by histamine. This, by table, is equivalent to 0.005125 gm. of histamine dichloride for the entire test liquid. From this, the amount of histamine dichloride previously found to be present in this sample of Witte's peptone—0.0001675 gm.—must be subtracted, which leaves 0.00496 gm. as the amount of the *introduced* histamine dichloride that was recovered, which is 99.3 per cent.

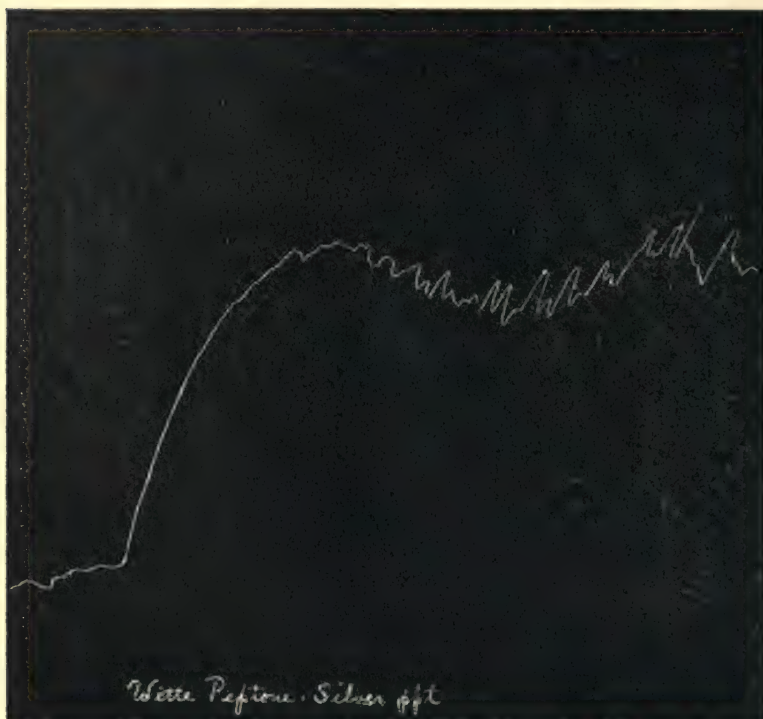


FIG. 4. Contraction of excised virgin guinea pig uterus produced by the silver precipitate from 1 gm. of Witte's peptone. The presence of histamine was also indicated by the colorimetric determination.

#### CONCLUSIONS.

1. A typical peptone shock is obtained by the injection of a histamine-free peptone; hence peptone shock and histamine shock are not identical.

2. A histamine-free peptone contracts the excised virgin guinea pig uterus.

3. Pure peptone contains a substance or substances, apparently basic in character, that are not destroyed by boiling with hydrochloric acid, that are precipitated by phosphotungstic acid, extracted by amyl alcohol from an alkaline aqueous solution, that combine with sulfuric acid to give salts that are soluble in water, and that are capable of producing a fall in blood pressure. This fall in blood pressure is, however, far inferior to the similar fall in blood pressure obtained with the equivalent quantity of peptone. In the above respects the substances are similar to histamine. There are, however, two very marked differences between the above substances and histamine; they give no color with *p*-phenyldiazonium sulfonate and they do not contract the excised virgin guinea pig uterus.

4. A sample of Witte's peptone—100 gm.—was found to contain the equivalent of 0.00335 gm. of histamine dichloride.





## STUDIES ON PROTEINOGENOUS AMINES.

### XI. RESPONSE OF THE EXCISED UTERUS TO POTASSIUM, RUBIDIUM, AND CESIUM IONS.

By MILTON T. HANKE AND KARL K. KOESSLER.

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(Received for publication, June 21, 1920.)

While we were searching for histamine in feces, blood, liver, and hypophysis, we noticed that the silver filtrate fractions,<sup>1</sup> although they could not have contained more than traces of histamine, frequently evoked a pronounced response from the excised virgin guinea pig uterus. Since this fraction always contains a small quantity of the nitrate ion, which is originally introduced as silver nitrate, we thought, at first, that this ion might be responsible for the physiological activity. To test the accuracy of this surmise, 1 cc. each of chemically pure 10 per cent solutions of sodium and potassium nitrate were separately introduced into the 100 cc. of Locke-Ringer solution in which the uterus was suspended. Fig. 1 illustrates the response obtained with the potassium nitrate solution. *A response was not obtained with the sodium nitrate solution.* From these results we concluded that the sodium and the nitrate ions did not stimulate the uterus muscle and that the response obtained with potassium nitrate was due to the potassium ion. This led naturally to an investigation of the effect of some of the other closely related metallic ions on the excised uterus.

Solutions of chemically pure sodium chloride, sodium nitrate, lithium chloride, ammonium chloride, potassium chloride, potassium nitrate, rubidium chloride, cesium chloride, calcium chloride, and magnesium sulfate were employed in these tests. *A response was obtained only with the potassium, rubidium, and*

<sup>1</sup> Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1920, xliii, 547.



FIG. 1. Response of the excised virgin guinea pig uterus to potassium nitrate. The uterus was suspended in 100 cc. of a Locke-Ringer solution.

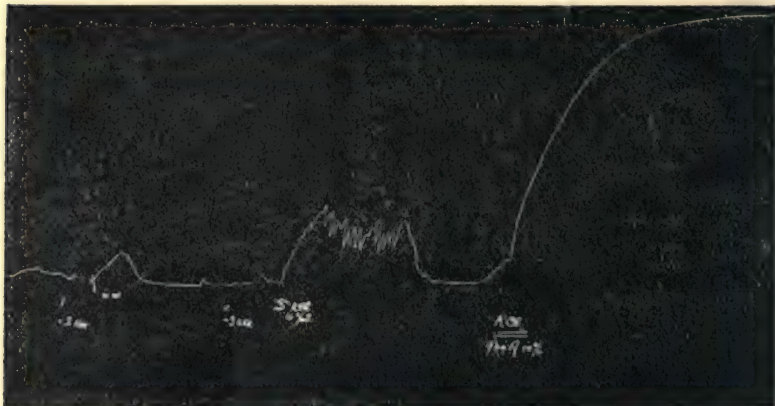


FIG. 2. Response of the excised virgin guinea pig uterus to various concentrations of potassium chloride. The uterus was suspended in 100 cc. of a Locke-Ringer solution.





FIG. 3. The response of the excised virgin guinea pig uterus to various concentrations of rubidium chloride. The uterus was suspended in 100 cc. of a Locke-Ringer solution.

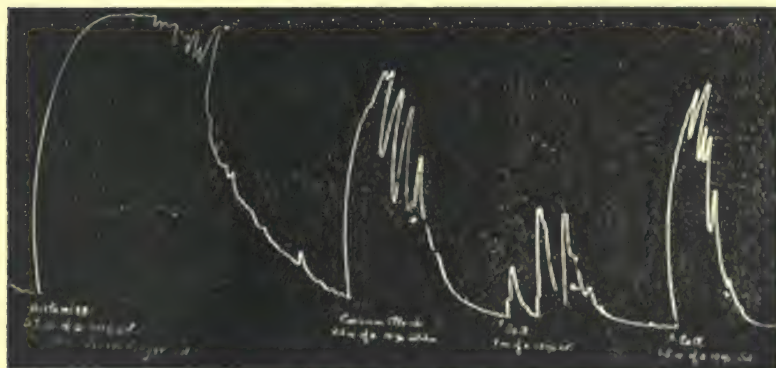


FIG. 4. Response of the excised virgin guinea pig uterus to various concentrations of cesium chloride. The uterus was suspended in 100 cc. of a Locke-Ringer solution. The first curve, a typical histamine tracing, is introduced for the sake of comparison.

*cesium salts.* Fig. 2 shows the response obtained when 0.20, 0.30, 0.50, and 1.0 cc. of a 10 per cent solution of potassium chloride were introduced into the 100 cc. of Locke-Ringer solution in which the uterus was suspended. Fig. 3 illustrates the response obtained when 0.05, 0.10, 0.40, 0.80, and 1.6 cc. of a 10 per cent solution of rubidium chloride were introduced into the 100 cc. of Locke-Ringer solution in which the uterus was suspended. The last two tracings in this figure illustrate the response evoked by equivalent quantities of potassium and rubidium salts. Fig. 4 shows the response obtained when 1.0, 1.5, and 2.2 cc. of a 10 per cent solution of cesium chloride were introduced into the 100 cc. of Locke-Ringer solution. The first tracing in this figure was obtained by introducing 0.50 cc. of a 0.01 per cent solution of histamine dichloride into the 100 cc. of Locke-Ringer solution and it is inserted here merely for comparison.

Since no response was obtained with equivalent quantities of sodium chloride, sodium nitrate, ammonium chloride, lithium chloride, calcium chloride, and magnesium sulfate it is safe to say that the Cl, NO<sub>3</sub>, SO<sub>4</sub>, NH<sub>4</sub>, Li, Ca, and Mg ions have no stimulating action upon the uterus muscle and that the potassium, rubidium, and cesium ions are responsible for the response obtained.

#### CONCLUSIONS.

Potassium and rubidium salts in sufficient concentration—about  $N/75$  in each case—produce a tonic contraction of the virgin guinea pig uterus from which the muscle does not recover until the stimulating ions are removed. The minimum effective concentration of the potassium ion is  $N/150$  and of the rubidium ion is  $N/1,210$  from which it appears that the rubidium is approximately eight times as active as potassium. The minimum effective concentration of cesium appears to be about  $N/150$ ; but the response obtained with this ion is neither so vigorous nor so permanent as that obtained with either potassium or rubidium.

## AN IMPROVED VOLUMETRIC PUMP FOR CONTINUOUS INTRAVENOUS INJECTIONS.

By R. T. WOODYATT.

(From the Otho S. A. Sprague Memorial Institute, Laboratory for Clinical  
Research, Rush Medical College, Chicago.)

Plate 3.

(Received for publication, January 16, 1920.)

A machine described earlier<sup>1</sup> consisted essentially of a single glass syringe fitted with a two-way valve, syringe and valve being operated by an electric motor acting through a worm gear, eccentrics, and rods. The discharge of the pump was controlled coarsely by setting the stroke of the piston by means of a system of levers and more exactly through control of the motor speed by means of a rheostat. This system involved the use of variable speed motors and changing motor speeds, with the inherent disadvantages which this implies from the standpoint of uniform performance.

The present machine mounts two glass syringes or cylinders each fitted with a two-way valve. Both pumps are run by one motor acting as before through a worm gear, eccentrics, etc. The former method of adjusting the stroke is displaced by a new device which is applied separately to each piston rod. The new device is simpler and much more accurate than the old and permits the stroke of either piston to be set independently in a few seconds at the desired length while the machine is running or stopped. Owing to the stroke adjustment it becomes unnecessary to alter the speed of the motor during an experiment, thus making it possible to drop the variable speed motor and rheostat in favor of a motor of constant speed type with the decided advantage that the operator's attention is not required to secure uniform performances. With a half-horse-power "Synchronous" motor, long experiments involving repeated changes in the rate of dis-

<sup>1</sup> Woodyatt, R. T., *J. Biol. Chem.*, 1917, xxix, 355.



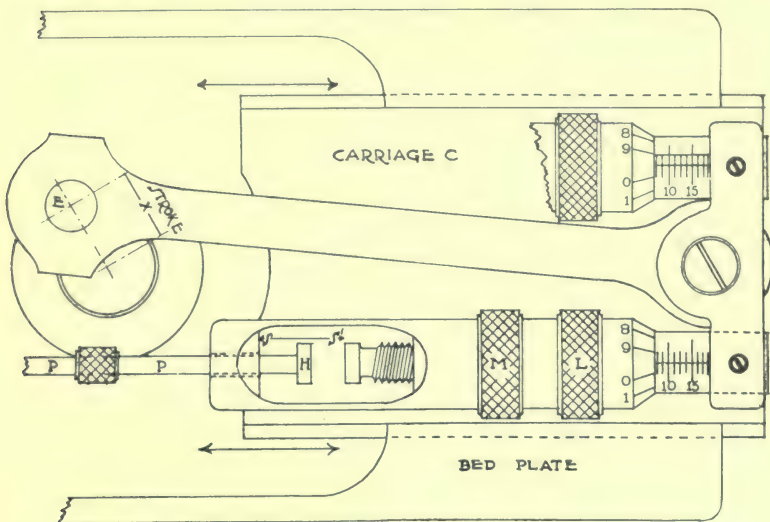
charge from one or both cylinders have been conducted easily and with negligible fluctuations of the motor speed.

The stroke adjustment is illustrated in Text-fig. 1. The motion of the motor is transmitted through a worm gear to the eccentric E and by the eccentric rod to a carriage plate C. The carriage C moves in a grooved bed plate in the direction of the arrows making a stroke equal to the stroke X of the eccentric. The diagram shows the piston rod P broken off at its point of emergence from the pump cylinder. The piston rod is jointed for convenience in taking the machine apart and terminates in a hard steel head H standing free in the space SS'. This space is the measuring gap in a machinist's micrometer modified for the purpose. The micrometer is fixed by a mounting on the right to the carriage itself and projects to the left over the plane of the carriage with clearance to permit of its operation. The distance SS' can be given any value desired by loosening the lock nut L and turning the milled collar M which causes the surface S to move toward or away from S'. When the machine is in motion it will be seen that the distance SS' can be made so great that after one complete stroke the head H will have been pushed as far to the left as the extreme forward motion of S' will permit, after which it will remain stationary, S' just touching it at the end of each subsequent forward stroke while on the back stroke S will merely approach H. But as the gap SS' is shortened by turning M so that S approaches S' a position may be found in which at the end of the back stroke S will also just touch H. With this setting of the micrometer, H is just touched but not moved on the forward stroke by S' and on the back stroke by S. This position corresponds to 0 on the scale and collar of the micrometer. The carriage now moves back and forth through the distance X while none of its motion is imparted to the piston although while the piston remains stationary the valves are turned. Now if S is set 1 cm. closer to S' as read directly on the scale and collar, then H will be displaced 1 cm. on the back stroke and on the forward stroke will return to its former position, and so on for any setting within the range of the apparatus. The micrometer reads to 0.04 mm.

It will be noted that with this device the head H and so the piston rod and piston are free during the interval after the surface



S' having pushed H to the limit is receding and while the surface S is approaching H but is not yet in contact with it. This interval follows immediately upon the completion of the systole of the pump when the pressure of the fluid in the discharge tube leading from the pump is highest. If sufficient back pressure develops it may kick back the piston before the valve cuts off the communication thus destroying the quantitative character of the pump and reducing the total discharge. There is a similar interval at the end of the diastole during which the pressure of the gravity feed



TEXT-FIG. 1. Improved volumetric pump for continuous intravenous injections. The actual length is 10 inches over all.

if such is used might move a loose piston and allow the cylinder to take in more than the indicated volume of fluid at each stroke. These sources of error are eliminated by a friction check on the piston rod which makes it impossible for the piston to move except in response to impacts of the surface S and S'.

The present machine has the advantages of two single machines of the earlier type in that it permits the simultaneous injection of one or two liquids into one or two discharge tubes, both constantly at different rates bearing known ratios, one constantly

at one rate with one varying, or both varying as desired. The accuracy and evenness of performance as well as the convenience of operation are much higher than in the older machine. Having a motor which runs always at the same speed, a cylinder (syringe) is calibrated by direct observation of the total volume which it discharges during periods of 15 minutes to 1 hour, with the stroke set at 0, at 15 mm., and at two or more intermediate points. In plotting the results they are found to fall on a straight line and the chart so formed indicates the setting of the micrometer necessary for the delivery of any desired volume per hour. When set to deliver a given volume in an hour, the results with the present motor fed from an ordinary power circuit have frequently fallen within 0.1 to 0.3 cc. of the desired total.

The machine was made by William Gaertner and Company, 5345 Lake Park Avenue, Chicago, Illinois, and is illustrated in Fig. 1.

#### EXPLANATION OF PLATE 3.

FIG. 1. Assembled machine. The syringes and valves are demountable without tools for cleansing and sterilization purposes. The large screw head at the left and below is at the end of the worm shaft bearing. The motor shaft is coupled to the opposite end of the worm shaft.

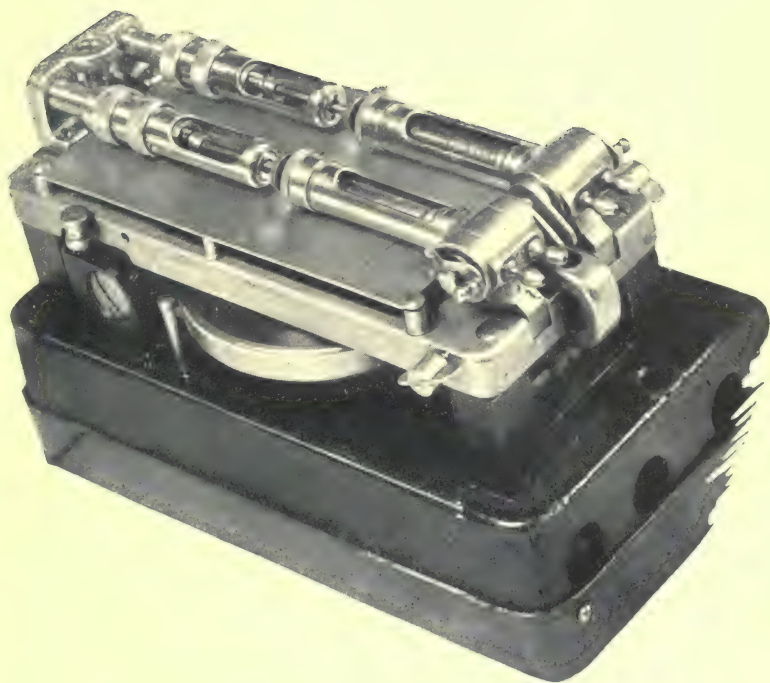


FIG. 1.

(Woodyatt: Pump for intravenous injections.)





## DISODIUM PHOSPHATE AS A CATALYST FOR THE QUANTITATIVE OXIDATION OF GLUCOSE TO CARBON DIOXIDE WITH HYDROGEN PEROXIDE.

By EDGAR J. WITZEMANN.

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Chicago.)

(Received for publication, July 13, 1920.)

The experiments described in this paper represent a confirmation and extension of part of Löb's observations on the influence of phosphates on oxidative glycolysis. By the experiments herein described it is proved that disodium phosphate catalyzes the quantitative oxidation of glucose to carbon dioxide by hydrogen peroxide. Additional experiments on the influence of the carbonates of sodium and other compounds are included and a partial interpretation of the results is offered.

Previous experiments on the influence of phosphates on the oxidation of butyric acid<sup>1</sup> with hydrogen peroxide were being extended by further experiments when it was realized that if the results of Löb and his coworkers,<sup>2,3</sup> on the influence of phosphates on glucose oxidation with peroxide, could be demonstrated by an adequate method the results would help clarify the influence of phosphates on butyric acid oxidation and have considerable interest in other ways. The results of some earlier work on the oxidation of glucose<sup>4</sup> indicated that probably the amount of oxidation observed by Löb could be exactly determined by the method suggested by those data. This was confirmed.

<sup>1</sup> Witzemann, E. J., *J. Biol. Chem.*, 1918, xxxv, 83.

<sup>2</sup> Löb, W., and Pulvermacher, G., *Biochem. Z.*, 1910, xxix, 316. Löb, W., and Gutmann, S., *Biochem. Z.*, 1912, xlv, 288. Beysel, W., and Löb, W., *Biochem. Z.*, 1915, lxviii, 368.

<sup>3</sup> Löb, W., *Biochem. Z.*, 1911, xxxii, 43.

<sup>4</sup> Witzemann, E. J., *J. Am. Chem. Soc.*, 1916, xxxviii, 150.

The statements under consideration as given in Löb's summary<sup>3</sup> are quite definite and are in part as follows:

"(1) In salt-free sugar solutions hydrogen peroxide produces only a vanishingly small amount of oxidative glycolysis.

(2) The glycolysis is markedly increased by raising the hydroxyl ion concentration.

(3) With the small OH ion concentrations in solutions having the alkalinity of blood, which is only slightly different from that of water, glycolysis is very slight if it is not accelerated by phosphates.

(4) The phosphate ions accelerate the glycolysis by the OH ions; the most favorable OH ion concentration within the limits tested lies at pH 8.302 to 7.070. At pH  $\geq$  about 5.600 there is no longer a perceptible OH ion effect exceeding that of pure water, even in the presence of phosphate ions.

(5) The acceleration of the glycolysis increases with constant OH ion concentration with increase in the absolute amount of phosphate added."

An examination of the experimental data, however, leaves one in doubt as to whether Löb really measured the oxidative glycolysis. In fact Michaelis and Rona<sup>5</sup> were not convinced by Löb's data and interpreted his observations differently. Obviously his determination of optical rotation and the reduction of Fehling solution by the glucose solutions before and after oxidation was not a determination of the absolute amount of oxidation. Consequently the term "oxidative glycolysis," which he uses to describe these phenomena, might include two processes.

(a) Destruction of glucose by oxidation at the expense of oxygen from the hydrogen peroxide used. This is what Löb meant.

(b) Destruction of glucose by intramolecular rearrangement under the influence of alkali. This kind of chemical change is what Michaelis and Rona appear to think Löb really saw at least in part.

If a neutral phosphate system such as Löb used, which is known to be a constituent of many living organisms, has any considerable effect upon oxidation the scope and nature of the effects should be known. The possible importance of such facts biologically for instance, when considered in relation to the well known indispensable relationships between phosphates and much normal cellular oxidation, is too obvious to require further comment.

<sup>5</sup> Michaelis, L., and Rona, P., *Biochem. Z.*, 1912, xlvii, 447.

The data described in this paper are sufficiently definite to give a new interest to the many facts already in hand in this field and to serve as a definite point of reference in the further study of these questions.

The results described here have a general interest in another way also. In the interpretation of the action of alkaline substances on sugars two points of view are recognized. According to the one the known effects of alkaline substances on sugars are due essentially to the hydroxyl ions. The other older view recognizes that the undissociated molecules and other ions may also aid or produce other effects than those of the so called hydroxyl ion effects. Without reviewing this problem any further in this paper it may simply be stated that the data herein presented offer varied and interesting support for the latter view.

#### EXPERIMENTAL.

Considering the importance of Löb's claims from several points of view it seemed highly important to determine accurately how much oxidation actually took place in his experiments with phosphates. This, it was thought, could be done, by applying the results of the author's previous study of the complete oxidation of glucose with potassium permanganate<sup>4</sup> to the analysis of the results obtained by Löb's experiments.

*1. Methods of Analysis.*—Previous experiments on the oxidation of glucose showed that in alkaline solution it is quantitatively oxidized to carbon dioxide and oxalic acid with potassium permanganate. The oxalic acid in turn is quantitatively oxidized to carbon dioxide by permanganate in sulfuric acid solutions. The plan was therefore as follows:

1. Oxidize glucose with hydrogen peroxide in the presence of phosphates just as Löb did.

2. After the expiration of the proper time interval add excess manganese dioxide to decompose unchanged hydrogen peroxide.

3. After decomposition is complete filter off the manganese dioxide, washing the filter and the original flask thoroughly.

4. Add excess sodium hydroxide solution.

5. Add an accurately known amount but excess of a strong accurately standardized solution of potassium permanganate



(about 3 gm. per 100 cc.). Heat this mixture to boiling and set aside over night after covering the top of the hot flask.

6. Add excess concentrated sulfuric acid.

7. Add an accurately known amount but excess of an accurately standardized solution (about 6 gm. per 100 cc.) of oxalic acid.

8. Dilute the clear colorless solution to a convenient definite volume and using an aliquot portion titrate back the excess oxalic acid with dilute potassium permanganate solution (0.1 N).

9. Calculate the total permanganate required for complete oxidation of the solution in No. 8, add the permanganate added in the beginning, and subtract the permanganate equivalent of the oxalic acid used. The result is the amount of permanganate utilized by the glucose or other incompletely oxidized compounds present and may easily be calculated to its glucose equivalent.

In order to test the accuracy of the above method a solution of pure glucose containing 10 gm. per liter was prepared. 20 cc. of this solution, containing 0.200 gm. of glucose, 15 cc. of 35 per cent sodium hydroxide solution, and 75 cc. (= 2.028 gm.) of potassium permanganate solution were heated to boiling. After standing over night excess concentrated sulfuric acid was added and then 50 cc. (= 1.519 gm. of  $\text{KMnO}_4$ ) of an oxalic acid solution. This colorless solution was diluted to 500 cc. in a graduated flask. 25 cc. portions were titrated back with 0.0996 N potassium permanganate. 5.70 cc. were required.  $5.70 \times 20 \times 0.003146 = 0.359$  gm. of  $\text{KMnO}_4$  required for the excess oxalic acid that was added.

2.028	gm. $\text{KMnO}_4$ originally added.
0.359	" $\text{KMnO}_4$ required for excess oxalic acid.
2.387	" $\text{KMnO}_4$ used (total).
1.519	" $\text{KMnO}_4$ equivalent of oxalic acid added.
0.868	" $\text{KMnO}_4$ reduced by the glucose.

Since 2.40 molecules of  $\text{KMnO}_4$  are required to oxidize 1 molecule of glucose to carbon dioxide the equation

$$\begin{aligned} 758.4:180 &= 0.868:x \\ x &= 0.206 \text{ gm. glucose} \end{aligned}$$

gives the amount of glucose originally present.

Another oxidation made at the same time gave 0.198 gm. of glucose.



These were the results obtained with the first pair of oxidations tried and give fairly the maximum analytical error as demonstrated by subsequent experience. The results show that the method will be satisfactory provided the amount of oxidation observed exceeds the experimental error of 2 or 3 per cent.<sup>6</sup>

*Experiments with the Phosphates of Sodium.*

2. *Repetition of Löb's Experiments.*—Having established the fact that it is possible to determine glucose quantitatively in the proposed way the author repeated and analyzed by the method described above a number of the experiments carried out by Löb.

In Table I the results obtained in five oxidations carried out at room temperature for just 1 week are given. The results are calculated as though the oxygen required by the unoxidized compounds in the solution was all consumed by unchanged glucose. This is almost certainly not entirely true but since the incompletely oxidized compounds are possibly a complex mixture, difficult to analyze,<sup>7</sup> it seemed permissible and correct for the purposes of comparison to calculate the permanganate consumed to glucose. The results show that the influence of the phosphates

<sup>6</sup> A similar method was developed by Greifenhagen and coworkers (Greifenhagen, W., König, J., and Scholl, A., *Biochem. Z.*, 1911, xxxv, 169), and was found sufficiently accurate in use by Levene and Meyer (Levene, P. A., and Meyer, G. M., *J. Biol. Chem.*, 1912, xii, 265). These results were discovered after the completion of my own work.

<sup>7</sup> On the basis of Löb's earlier work (Löb, W., *Biochem. Z.*, 1908, xii, 78, 466; 1909, xvii, 132. Löb, W., and Pulvermacher, G., *Biochem. Z.*, 1909, xvii, 343. Löb, W., *Biochem. Z.*, 1909, xx, 516; xxii, 103; 1910, xxiii, 10; xxvi, 231), but specifically on the basis of a later statement (Löb, W., *Biochem. Z.*, 1915, lxxviii, 368) it might be concluded that the incompletely oxidized compounds are formic and polyhydroxy acids arising from formaldehyde and pentoses. Tests on solutions from complete oxidations known to reduce permanganate equivalent to 0.02 to 0.04 gm glucose in 75 cc. gave distinct tests for sugar with Haines' or Fehling's solution. Since this is near the limits of sensitiveness of these reagents, it appears that no large proportion of intermediate oxidation products (between hexose and CO<sub>2</sub>) can be present. This appears to conform with the observations of Smolka (Smolka, A., *Sitzungsb. Math. Natur. Akad. Wiss.*, 1887, xcv, pt. ii, 5) on the oxidation of glucose with insufficient neutral permanganate, who recovered only final oxidation products (HCO<sub>2</sub>H, H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>, and CO<sub>2</sub>) and the calculated amount of unchanged glucose.

is progressively greater with increasing concentration, but that it is not a linear function of the concentration since the relative acceleration diminishes with increasing phosphate concentration.

The results in Table II were obtained under exactly the same conditions as those in Table I except that the solutions were kept 98 hours (4 days, 2 hours) in an incubator at 37°C.

TABLE I.

*Glucose + H<sub>2</sub>O<sub>2</sub> + Phosphates at Room Temperature.*

20 cc. glucose solution (0.200 gm.) + 20 cc. 3 per cent H<sub>2</sub>O<sub>2</sub> in total volume of 75 cc.

No.	0.33 M Na <sub>2</sub> HPO <sub>4</sub> .	0.33 M NaH <sub>2</sub> PO <sub>4</sub> .	H <sub>2</sub> O	Reaction.	Glucose recovered.	Glucose oxidized.
	cc.	cc.	cc.	pH	gm.	per cent
1	0.0	0.0	35	7.07	0.2006	0.00
2	1.6	0.4	33	7.347	0.1863	6.85
3	6.4	1.6	27	7.347	0.1210	39.50
4	16.0	4.0	15	7.347	0.0658	67.10
5	25.6	6.4	3	7.347	0.0442	77.90

TABLE II.

*Glucose + H<sub>2</sub>O<sub>2</sub> + Phosphates at 37°.*

20 cc. glucose solution (0.200 gm.) + 20 cc. 3 per cent H<sub>2</sub>O<sub>2</sub> in total volume of 75 cc.

No.	0.33 M Na <sub>2</sub> HPO <sub>4</sub> .	0.33 M NaH <sub>2</sub> PO <sub>4</sub> .	H <sub>2</sub> O	Reaction.	Glucose recovered.	Glucose oxidized.
	cc.	cc.	cc.	pH	gm.	per cent
1	0.0	0.0	35	7.07	0.186	6.50
2	1.6	0.4	33	7.347	0.1163	41.85
3	6.4	1.6	27	7.347	0.019	90.50*
4	16.0	4.0	15	7.347	0.034	83.00
5	25.6	6.4	3	7.347	0.035	82.50

\* In the experiments at 37° it was generally observed that oxidation was less complete in No. 5 than in Nos. 3 or 4. Special experiments to interpret this apparent anomaly have not been done but the effect appears to be due to the fact that the velocity of oxygen activation by the Na<sub>2</sub>HPO<sub>4</sub> is greater than the velocity of oxygen consumption and consequently the excess active oxygen is lost as such from the reaction mixture. This interpretation is so far supported by facts given in this paper and by others not mentioned.

3. *Fate of the Glucose.*—The results given above prove quite conclusively that the glucose is oxidized. Of the large number of compounds into which it could conceivably be converted without oxidation only a small number are not completely oxidizable to carbon dioxide by permanganate in acid or alkaline solution.<sup>8</sup> Nevertheless it seemed necessary to demonstrate actually that carbon dioxide was an important product of this oxidation.

All the experiments on carbon dioxide recovery were done with mixtures corresponding to No. 5 in Tables I and II. In determining the CO<sub>2</sub> the oxidation mixture was placed in a round bottom flask attached to a reflux condenser and arranged so that CO<sub>2</sub>-free air could be bubbled through the mixture and then passed through wash bottles containing clear barium hydroxide solution.<sup>9</sup> On warming the flask nearly all the CO<sub>2</sub> was driven over. Excess dilute sulfuric acid was finally added and the mixture heated to boiling.

A. 75 cc. of such a solution, which had been kept in the incubator until all peroxide was gone and in which oxidation was nearly complete, gave in the CO<sub>2</sub> apparatus 0.13 gm. of barium carbonate or about 10 per cent of the calculated CO<sub>2</sub> yield. The rest of the CO<sub>2</sub> had been lost into the air.

B. 75 cc. of such a solution after 10 days at room temperature gave 0.69 gm. of BaCO<sub>3</sub>, equivalent to 0.154 gm. of CO<sub>2</sub> or a 52.6 per cent yield of CO<sub>2</sub>. The solution, to which excess sulfuric acid had been added while in the CO<sub>2</sub> apparatus, was alkalized with sodium hydroxide, treated with MnO<sub>2</sub> to remove unchanged peroxide, filtered, and treated as usual with permanganate. The permanganate consumed was equivalent to 0.0749 gm. of glucose or 37.5 per cent recovered.  $52.6 + 37.5 = 90.1$  per cent of the 0.200 gm. of glucose used recovered in this way.

Results similar to this were obtained under the same conditions a number of times.

<sup>8</sup> It was not until the experiments described above had been completed that it was suspected that oxidation to CO<sub>2</sub> was nearly quantitative. Löb expressed the opinion that formic and polyhydroxy acids are the main products and there was no reason to doubt this until the small amount of permanganate required to complete the oxidation suggested that the oxidation might already be largely completed to CO<sub>2</sub>.

<sup>9</sup> Evans, W. L., and Witzemann, E. J., *J. Am. Chem. Soc.*, 1912, xxxiv, 1086.



C. In order to obtain a more complete conversion into  $\text{CO}_2$  and a good recovery the oxidation was set up in the incubator. Two strong round bottom 300 cc. flasks, one of which contained barium hydroxide solution and the other the glucose oxidation mixture, were connected by a glass tube having two right angle bends in rubber stoppers. The stoppers were wired in and then covered over with molten paraffin. The whole was placed in the incubator at  $37^\circ\text{C}$ . and agitated a few moments every day for a week. It was then taken out and allowed to stand at room temperature several days with occasional agitation. The oxidation mixture gave 0.28 gm. of  $\text{BaCO}_3$  in the  $\text{CO}_2$  apparatus. The attached  $\text{Ba}(\text{OH})_2$  flask gave 0.83 gm. of  $\text{BaCO}_3$ . This corresponds to 0.2474 gm. of  $\text{CO}_2$  altogether or an 84.4 per cent yield of  $\text{CO}_2$ . The oxidation mixture treated as in (B) reduced permanganate equivalent to 0.0195 gm. of glucose or 9.8 per cent of the glucose used.  $84.4 + 9.8 = 94.2$  per cent of the glucose recovered in this way.

On the basis of these results there can be no doubt that the glucose unaccounted for by the permanganate consumed is really oxidized to  $\text{CO}_2$ .

In developing the above proof that practically quantitative oxidation to  $\text{CO}_2$  is obtained several other facts were observed.

1. The carbon dioxide formed is freely and easily lost from the solution during the oxidation even at the room temperature. In this the oxidation resembles vital oxidation in which the carbon dioxide is spontaneously lost during respiration. As much as two-thirds or more of the carbon dioxide obtained is evolved and absorbed by barium hydroxide in a closed apparatus at  $37^\circ\text{C}$ .

Ordinary alkaline oxidation systems, although they undergo changes in many ways similar to those occurring in living organisms, differ in that the  $\text{CO}_2$  formed is bound and held in the system as carbonate or bicarbonate. This easy formation and loss of  $\text{CO}_2$  is probably the most important physical characteristic of a vital oxidation system. It is not yet certain to what extent the phosphate systems can carry out the other functions belonging to alkaline systems, that are so important in the non-oxidative transformations of sugar in organisms, but indications are not lacking that they can also aid in some of these changes under suitable conditions.



2. The solutions in which all hydrogen peroxide had disappeared and which contained material oxidizable by permanganate equivalent to only 0.02 to 0.04 gm. of glucose in 75 cc. (*i.e.*, 0.027 to 0.053 per cent) reduced Fehling solution distinctly. Since this is close to the limits of sensitiveness of this test with pure glucose it is clear that most of the glucose attacked had been completely burned to carbon dioxide, and that no appreciable quantity of intermediate products such as polyhydroxy acids could be present.

4. *Influence of Additional Glucose and Peroxide.*—On the basis of the results in the preceding section it was of considerable interest to know whether the same phosphate mixture would repeatedly catalyze the oxidation of glucose. In other words whether the products of the reaction in any way "poison" the catalyst. If  $\text{CO}_2$  is the sole final product and if it is evolved as was shown above, the phosphate mixture should serve repeatedly in this oxidation just as it is known to do in the fermentation of glucose.<sup>10</sup>

Experiment 5, Table II, was set up in the incubator. After 3 days it was free from peroxide. 0.20 gm. of glucose and 20 cc. of 3 per cent peroxide were again added. After 1 week in the incubator the peroxide had again disappeared. The same materials were again added. After another week this was repeated. On determining the permanganate consumed in the usual way it was found to correspond to 0.0831 gm. of glucose. Since 0.80 gm. of glucose had been used this corresponds to 10.4 per cent of the glucose used, which is about what is recovered from a single experiment of this kind.

These results show that the functional activity of the disodium phosphate is not impaired in the catalysis. Since this does not occur it is clear that the disodium salt is not changed into monosodium phosphate by the carbonic acid, nor any other acid intermediate oxidation product, to any marked extent. If sodium bicarbonate were formed in this way the oxidation would be retarded or stopped in the typical way in which this compound acts (*cf.* Section 7).

<sup>10</sup> Harden, A., and Young, W. J., *J. Chem. Soc.*, 1905, xxi, 189; *Proc. Roy. Soc. London, Series B*, 1906, lxxvii, 405; 1908, lxxx, 299; 1909, lxxxi, 336. Young, W. J., *Proc. Roy. Soc. London, Series B*, 1909, lxxxi, 529. Harden, A., and Young, W. J., *Biochem. Z.*, 1911, xxxii, 173. Young, W. J., *Biochem. Z.*, 1911, xxxii, 177.

5. *Influence of Changing the Ratio of the Phosphates.*—The results in Table III constitute a repetition of part of Löb's experiments (Table XI)<sup>3</sup> on the influence of a change in the ratio of the two phosphates. All the experiments were set up in 250 cc. Florence flasks and kept in the incubator for 45 hours before analyzing.

TABLE III.

25 cc. (0.25 gm.) glucose + 25 cc.  $\text{H}_2\text{O}_2$  + 20 cc. salt solution + 5 cc. water at  $37^\circ$ .

No.	0.33 M $\text{Na}_2\text{HPO}_4$ .	0.33 M $\text{NaH}_2\text{PO}_4$ .	$\text{H}_2\text{O}$	Reaction.	Glucose recovered.	Glucose oxidized.
	cc.	cc.	cc.	pH	gm.	per cent
1	0	0	25	7.07	0.2407	3.7
2	16	4	5	7.347	0.1992	20.3
3	10	10	5	6.813	0.2068	17.3
4	4	16	5	6.239	0.2316	7.4
5	2	18	5	5.910	0.2342	6.3

TABLE IV.\*

20 cc. glucose (0.200 gm.) + 20 cc. 3 per cent  $\text{H}_2\text{O}_2$  at  $37^\circ$  for 10 days.

No.	0.33 M $\text{Na}_2\text{HPO}_4$ .	0.33 M $\text{NaH}_2\text{PO}_4$ .	$\text{H}_2\text{O}$	Glucose recovered.	Glucose recovered after 2 days.
	cc.	cc.	cc.	gm.	gm.
1	25.6	6.4	59	0.0242	0.0387
2	25.6	32.0	34	0.0180	0.0271
3	25.6	64.0	2	0.0166	

\* All the experiments in this table have a total volume of 131 cc. The reaction of No. 1 is distinctly alkaline to litmus paper while that of No. 3 is distinctly acid. Accordingly pH passes from a point on the alkaline side (about 7.347) to a point decidedly on the opposite or acid side of neutrality.

The results show a diminishing velocity of glucose oxidation as the ratio of monosodium phosphate used increases or as the ratio of disodium phosphate decreases.

From these experiments alone it might be concluded that the OH ion is significant in this oxidation but results given in the next paragraph do not confirm this idea.

When the ratio of the two phosphates is changed by changing the amount of monosodium phosphate but keeping the disodium phosphate constant in amount different results are obtained.

The results in Table IV show that in the presence of a constant amount of disodium phosphate increasing amounts of monosodium phosphate do not retard the oxidation of glucose. In fact the presence of the monosodium phosphate seems to facilitate the completion of the glucose oxidation in spite of the fact that relatively No. 3 is comparable with No. 4 in Table III as far as the proportion of the two phosphates is concerned. Exactly the same result was obtained with Nos. 1 and 2 when they were allowed to react only 2 days. No velocity experiments have been made to determine whether the excess  $\text{NaH}_2\text{PO}_4$  retards the oxidation as it does the evolution of  $\text{O}_2$  from  $\text{H}_2\text{O}_2$  but the results as given indicate that it does not.

It seems clear that if Löb had done these experiments, as well as some others described below, he would have found it impossible to ascribe so much influence to the  $\text{OH}$  ions in this catalysis, as he did.

6. *Influence of Time on the Oxidation.*—In order to follow the glucose oxidation from day to day a large experiment containing

TABLE V.

*Glucose.*

			gm
	At beginning contained.....		0.200
(a)	After 24 hrs. " .....		0.1869
(b)	" 48 " " .....		0.1611
(c)	" 72 " " .....		0.1551*
(d)	" 96 " " .....		0.1212
(e)	" 120 " " .....		0.1173*
(f)	" 168 " " .....		0.0823

\* When the results described in this table are plotted the two values marked with the asterisk lie considerably outside the curve. This is due to the fact that undecomposed hydrogen peroxide was still present when the potassium permanganate was added. Thus when two solutions containing exactly the same amount of glucose but one of which also contained 5 cc. of 3 per cent hydrogen peroxide were analyzed, without decomposing the peroxide, the former was found to contain 0.1802 gm. of glucose by the complete oxidation method. The other containing the peroxide apparently contained 0.2165 gm. of glucose when calculated on the basis of the oxygen consumed. This difference is due to the well known fact that hydrogen peroxide reduces permanganate with the evolution of oxygen.

The results are given in this form in order to illustrate this error.



six times as much material as No. 2 in Table II was set up and placed in the incubator at 37°. 75 cc. of this solution (corresponding to 0.200 gm. of glucose) were taken out for analysis at definite intervals as indicated in Table V.

*Experiments with the Carbonates of Sodium.*

The preceding results clearly confirm Löb's claim that in the presence of phosphate mixture glucose is oxidized by hydrogen peroxide. Since he failed to observe appreciable amounts of oxidation when he used the other common reaction regulator mixtures it seemed unnecessary to test these again for the present. It did seem advisable, however, to make some experiments with the carbonates of sodium for several obvious reasons.

7. *Influence of Sodium Carbonate-Bicarbonate.*—If the phosphates do not exercise a catalytic effect in this oxidation and the effect observed is due to OH ions then an equimolecular amount of sodium carbonate and bicarbonate should have fully as much effect. That this is not true was definitely established by the following experiment in which 2.43 gm. of  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ , 0.72 gm. of  $\text{NaHCO}_3$ , 35 cc. of distilled water, 20 cc. (0.200 gm.) of glucose solution, and 20 cc. of 3 per cent  $\text{H}_2\text{O}_2$  were kept 4 days at 37°. Upon analysis the peroxide was found to have been completely decomposed and equivalent of 0.1902 gm. of glucose was recovered; *i.e.*, 5 per cent was apparently oxidized as against 80 per cent oxidized with the corresponding phosphate mixture.

In the above experiment the two carbonates were used in the same molecular amounts and proportion as the two phosphates in Experiments 5 in Tables I and II. The solution therefore contained at least the same amount of available alkali but had a somewhat higher OH ion concentration than the phosphate mixture referred to. If OH ion concentration and available alkali are the controlling factors in these oxidations this experiment should have shown as much or more oxidation than was obtained with the phosphate mixture.

8. *Influence of  $\text{H}_2\text{CO}_3$  and  $\text{NaHCO}_3$ .*—The results in Table V suggest that the velocity of decomposition of sodium bicarbonate, possibly produced in the oxidation, may be a factor in determining the velocity of oxidation. The following three experiments were



done in order to test the influence of this condition. The experiments were set up in similar 250 cc. flasks and kept in the incubator for 24 hours at 37° after which they were analyzed in the usual manner.

(1) 32.0 cc. of 0.33 M  $\text{NaH}_2\text{PO}_4$  solution + 1.22 gm. of  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ . This mixture effervesced in the cold. It was heated to boiling to expel  $\text{CO}_2$ , cooled, and the following were added:

20 cc. (0.200 gm.) of glucose solution, 20 cc. of 3 per cent  $\text{H}_2\text{O}_2$ , 3 cc. of distilled water. 0.0761 gm. of glucose was recovered.

(2) Components the same as in (1).

All ingredients were mixed except the peroxide before adding the  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$  in order to prevent the loss of  $\text{CO}_2$  as much as possible. 0.1906 gm. of glucose was recovered.

(3) 25.6 cc. of 0.33 M  $\text{Na}_2\text{HPO}_4$  solution.

6.4 " " 0.33 "  $\text{NaH}_2\text{PO}_4$  "

20.0 " (0.200 gm.) of glucose solution.

20.0 " of 3 per cent  $\text{H}_2\text{O}_2$ .

3.0 " " distilled water.

0.0774 gm. of glucose was recovered.

On the basis of the conditions of the experiments the results of (1) and (3) were expected to be identical because the reaction mixtures as used were identical. As a matter of fact the amount of glucose recovered was nearly the same in (1) and (3). It was expected that the oxidation in (2) would be somewhat slower. In fact only 5 per cent of the glucose was oxidized in (2) as compared with over 60 per cent in the others. This indicates that not only do  $\text{H}_2\text{CO}_3$  and  $\text{NaHCO}_3$  not catalyze the oxidation of glucose with hydrogen peroxide but that they actually retard it.

The interpretation of the influence of the sodium carbonate added in (2) has not been fully established as yet. There are several factors to be considered, three of which are as follows: (a)  $\text{Na}_2\text{CO}_3$  may under the conditions in (2) not react completely to give only  $\text{Na}_2\text{HPO}_4$  and  $\text{H}_2\text{CO}_3$ ; (b) if so, any  $\text{Na}_2\text{CO}_3$  or  $\text{NaHCO}_3$  remaining would rapidly catalytically decompose the  $\text{H}_2\text{O}_2$ ; (c) the presence of  $\text{CO}_2$  to the point of supersaturation may retard the activation or dissociation of  $\text{H}_2\text{O}_2$ .

9. *Influence of Sodium Carbonate.*—The following experiments were done in order to determine what influence sodium carbonate exercises on the action of disodium phosphate.

(1) 32.0 cc. of 0.33 M  $\text{Na}_2\text{HPO}_4$  solution.

3.0 " " water.

20.0 " " glucose solution (0.200 gm.).

20.0 " " 3 per cent hydrogen peroxide.

0.0311 gm. of glucose was recovered.

(2) Same as in (1) with 0.61 gm. of  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ .

0.1741 gm. of glucose was recovered.

(3) Same as (1) with 1.22 gm. of  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ .

0.1737 gm. of glucose was recovered.

The solutions were kept in the incubator at  $37^\circ\text{C}$ . for 45 hours and on analysis the amounts of unchanged glucose given were found. All hydrogen peroxide present had been decomposed.

The results show that sodium carbonate exercises a strongly negative influence on this oxidation in spite of the fact that the OH ion concentration is higher in (2) and (3) and the available alkali in (3) is twice what it was in Experiments 5, Tables I and II.

This negative influence on the final result of the oxidation may be due simply to the fact that the velocity of decomposition of peroxide by  $\text{Na}_2\text{CO}_3$  is many times greater than that by  $\text{Na}_2\text{HPO}_4$  and that the glucose oxidation induced by  $\text{Na}_2\text{CO}_3$  itself is relatively small in comparison.

The above observations on the influence of the carbonates of sodium permit us to conclude that, whatever the mechanism of  $\text{CO}_2$  formation in this oxidation may be, carbonates of sodium are not intermediate stages in the process of  $\text{CO}_2$  liberation.

10. *Influence of Sodium Hydroxide.*—The following three experiments were done in order to determine the influence of sodium hydroxide on the effect of the phosphate mixture.

(1) 25.6 cc. of 0.33 M  $\text{Na}_2\text{HPO}_4$  solution + 6.4 cc. of 0.33 M  $\text{NaH}_2\text{PO}_4$  + 20 cc. (0.200 gm.) of glucose solution + 20 cc. of 3 per cent  $\text{H}_2\text{O}_2$  + 3 cc. of water.

(2) The same as (1) except that one-half the water was replaced with 1.5 cc. (0.0857 gm.) of NaOH solution.

(3) The same as (1) except that all the water was replaced with 3 cc. (0.1714 gm.) of NaOH solution.

After 50½ hours at 37°C. the solutions contained no unchanged peroxide. They were analyzed and found to reduce  $\text{KMnO}_4$  corresponding to glucose as follows:

- (1) 0.0088 gm. of glucose.
- (2) 0.0140 " " "
- (3) 0.0618 " " "

The sodium hydroxide has a perceptible but not a large retarding effect which is interpreted tentatively in the light of other experiments as due simply to its effect in increasing the decomposition of the hydrogen peroxide. In this respect its activity is not so great as that of the carbonates which coincides with its smaller retarding effect on the action of the phosphate mixture.

*11. Partial Interpretation of the Influence of Disodium Phosphate.*—Since there are three compounds actively concerned in this oxidation reaction and since glucose and peroxide alone do not react appreciably there remain three possible ways of interpreting the reaction on the basis of the formation of molecular complexes which are so frequently found to underlie catalytic phenomena.

(1)  $\text{Na}_2\text{HPO}_4$  and  $\text{H}_2\text{O}_2$  may give an unstable complex which in turn reacts to oxidize glucose.

(2)  $\text{Na}_2\text{HPO}_4$  and glucose may form a hexose phosphate which is more sensitive to  $\text{H}_2\text{O}_2$  than free glucose.

(3) The three compounds may form a single complex the instability of which gives rise to the oxidation.

(1) and (2) are readily capable of being tested experimentally by known methods. (3) could conceivably take place in several ways none of which appears to be readily capable of experimental confirmation.

*The  $\text{Na}_2\text{HPO}_4$ - $\text{H}_2\text{O}_2$  Complex.*—That such a complex may be formed is suggested by the experiments of Petrenko on  $\text{H}_2\text{O}_2$  derivatives of  $\text{Na}_3\text{PO}_4$ .<sup>11</sup> A perphosphate of  $\text{Na}_2\text{HPO}_4$  is unknown. Moreover perphosphoric acid is apparently unknown.<sup>12</sup> However, pyrophosphoric acid gives a peracid with  $\text{H}_2\text{O}_2$ , stronger

<sup>11</sup> Petrenko, G., *J. russ. phys.-chem. Ges.*, 1902, xxxiv, 204, 391; *Chem. Zentr.*, 1902, i, 1263; ii, 95. Cf. also, Gemlin-Kraut, *Handbuch der anorganische Chemie*, Heidelberg, 7th edition, 1906, i, pt. 1, 146.

<sup>12</sup> Price, T. S., *Per acids and their salts*, New York, 1912, 77.



than Caro's acid and which oxidizes Mn to  $\text{KMnO}_4$  and its sodium salt  $\text{Na}_4\text{P}_2\text{O}_7$  gives a stable persalt with 3 per cent  $\text{H}_2\text{O}_2$ .<sup>13</sup>

There is therefore some basis in fact, even in this little studied field, for the idea that  $\text{Na}_2\text{HPO}_4$  may form an unstable perphosphate as is suggested in the succeeding paragraphs.

*Decomposition of Hydrogen Peroxide by the Phosphate Mixture.*—Various experiments were done on the influence of  $\text{Na}_2\text{HPO}_4$  on hydrogen peroxide although it is definitely stated<sup>14</sup> that it is without influence on peroxide. My own experiments, which will not be described here, show that it does decompose hydrogen peroxide and that the presence of an equimolecular amount of  $\text{NaH}_2\text{PO}_4$  retards but does not stop the decomposition.

TABLE VI.  
*0.1 N  $\text{KMnO}_4$  Consumed by 5 Cc. of the Mixture.*

No.	23.5 hrs.	47.2 hrs.	96.2 hrs.
	cc.	cc.	cc.
1	22.70	22.62	22.45
2	22.10	21.06	18.45
3	19.35	14.53	2.75
4	17.06	11.65	4.31
5	15.72	9.86	4.08

The only experiments to be described here represent a repetition of the glucose oxidations at  $37^\circ$  in Table II in which the glucose was omitted and in which the peroxide content was determined at intervals during 96 hours. The results given in Table VI indicate the amount of peroxide remaining, at the various intervals, in terms of cc. of 0.1 N  $\text{KMnO}_4$  consumed by 5 cc. of the mixture.

The phosphate mixtures and the hydrogen peroxide were warmed for 24 hours at  $37^\circ\text{C}$ . before being mixed in order to prevent a lag which is otherwise observed during the first 24 hours.

<sup>13</sup> Schenck, R., Vorländer, F., and Dux, W., *Z. angew. Chem.*, **1914**, xxvii, pt. 1, 291.

<sup>14</sup> Gemelin-Kraut,<sup>11</sup> p. 137.



The data show a progressively increasing decomposing effect with increasing phosphate content although the mixture is neither appreciably acid nor alkaline.<sup>15</sup>

In conclusion it may be stated that there are clear indications that  $\text{Na}_2\text{HPO}_4\text{-H}_2\text{O}_2$  may form an unstable complex, but as yet there is no satisfactory evidence.

*The  $\text{Na}_2\text{HPO}_4$ -Glucose Complex.*—Harden and Young, von Lebedew, and others<sup>10,16</sup> showed that, in the yeast fermentation of glucose,  $\text{Na}_2\text{HPO}_4$  combines with glucose to form a hexose phosphate ester. The presence of this complex was demonstrated in part by the fact that much of the phosphate was no longer precipitated with "magnesia mixture." Other hexose phosphoric esters have been obtained by chemical methods<sup>17</sup> but the laboratory preparation of von Lebedew,<sup>16</sup> and the commercial manufacture<sup>18</sup> of hexose phosphate ester are carried out only in the presence of growing yeast. It therefore seemed necessary to determine experimentally whether such a complex is formed in aqueous solutions of glucose and the phosphate mixture alone.

A solution corresponding to No. 5 in Table II except that it contained 20 cc. of water instead of the peroxide solution was kept 3 weeks in the incubator at 37°C. At the end of this time the glucose content was found by reduction methods to be unchanged. After standing 3 weeks more in the laboratory the

<sup>15</sup> It should be noted that the interpretation of this decomposition of  $\text{H}_2\text{O}_2$  in this case cannot be attributed to the OH ion, since the solution has about the OH ion concentration of water, which is without influence. It is interesting to note in this connection that Schenck, Vorländer, and Dux<sup>12</sup> found that  $\text{Na}_4\text{P}_2\text{O}_7$  solutions, which are so alkaline as to feel "soapy," actually stabilize  $\text{H}_2\text{O}_2$  by forming a stable perpyrophosphate. Moreover, it was found in experiments which will not be given here that the presence of  $\text{Na}_4\text{P}_2\text{O}_7$  with  $\text{Na}_2\text{HPO}_4$  retards or prevents the oxidation of glucose with  $\text{H}_2\text{O}_2$ , but not by decomposing the  $\text{H}_2\text{O}_2$  as with  $\text{NaHCO}_3$  or  $\text{Na}_2\text{CO}_3$ .

<sup>16</sup> von Lebedew, A. V., *Biochem. Z.*, 1910, xxviii, 213; 1911, xxxvi, 248. Embden, G., and Laquer, F., *Z. physiol. Chem.*, 1914-15, xciii, 94. Embden, G., Griesbach, W., and Laquer, F., *Z. physiol. Chem.*, 1914-15, xciii, 124.

<sup>17</sup> Cf. foot-note, Meyer, V., and Jacobson, P., *Lehrbuch der organischen Chemie*, Leipsic, 2nd edition, 1902, ii, pt. 2, 927.

<sup>18</sup> Cf. for instance Bayer and Company, German Patent 292,817, February 26, 1915; *Chem. Abstr.*, 1917, xi, 1519.

phosphate was precipitated with "magnesia mixture" and weighed as the pyrophosphate. A solution of the phosphates alone made up to the same volume was similarly precipitated at the same time. The two precipitates after ignition showed the same weight, within a small fraction of 1 per cent, which shows that hexose phosphate ester was not formed to any significant extent.

Similar solutions containing glucose and the phosphate mixture were kept under observation in the polariscope in comparison with glucose solutions without phosphates. In 4 days there was no measurable change in optical rotation in either solution.

These data, taken with the absence of positive data in the literature, seem to prove that a hexose phosphate ester such as was found by Harden and Young is not formed under these conditions and consequently has no part in bringing about this oxidation of glucose. If some other type of complex is formed its presence was not demonstrated by these methods.

*Influence of Time on the Glucose-Phosphate-Peroxide Reaction.*—When it was found that the rate of peroxide decomposition has a definite relation to the phosphate concentration it was of interest to learn what relation the rate of glucose oxidation bears to the rate of peroxide decomposition. The results given in Table VII are typical for the rate of glucose oxidation as obtained by compiling experimental results obtained in conditions like those used for Table II.

In order to test this more fully experiments like Nos. 1 to 5 in Table II were set up having a total volume of 150 cc. and which were placed in the incubator at 37°. The phosphate-glucose mixture and the peroxide were warmed separately for 24 hours before mixing to eliminate what appeared to be a temperature lag in the curves. 10 cc. were removed and analyzed at definite intervals and the results were calculated and recorded in Table VIII on the basis of 75 cc. and thus correspond to the results in Table VII. The materials used in Table VII were not warmed before mixing which accounts for the difference in the slope of the curves when the data are plotted.

The results in both series are substantially the same and show that the rate of glucose disappearance in the presence of the phosphate mixture is appreciably faster than the rate of  $\text{H}_2\text{O}_2$  disappearance in the absence of glucose (Table VI).

The above constitutes a partial experimental interpretation of the catalytic influence of disodium phosphate on the oxidation of glucose with hydrogen peroxide. The results clearly suggest that the glucose is really oxidized by an unstable disodium perphosphate, formed by the action of peroxide on disodium phosphate.

TABLE VII.  
*Glucose Remaining from 0.200 Gm. Used.*

No.	50 hrs.	97 hrs.
	<i>gm.</i>	<i>gm.</i>
1		0.1897
2		0.1843
3		0.1249
4	0.1037	0.0135
5	0.0088	

TABLE VIII.  
*Glucose Remaining from 0.1902 Gm. Used.*

No.	26.5 hrs.	49.2 hrs.
	<i>gm.</i>	<i>gm.</i>
1	0.1702	0.1455
2	0.1362	0.1074*
3	0.0489	
4	0.0195	
5	0.0273	

\* The behavior of No. 2 is quite variable. Sometimes oxidation is as slow as in No. 1 without phosphates and sometimes it is nearly as fast as in No. 3, but more frequently it is about as given in these results.

12. *Is a Glucose-Phosphate Solution Oxidized by Air?*—Having shown that the disodium phosphate plays a specific rôle in this catalysis the question arises as to whether the use of peroxide is necessary. A few experiments were done in order to determine whether air could be used instead of peroxide. It is well known that caustic alkalies catalyze the oxidation of sugars by air, with the formation of more or less  $\text{CO}_2$  depending on the conditions of the experiment. In the absence of definite data it was possible that the phosphate mixture might play the rôle of caustic



alkali. A mixture like No. 5 in Table II was placed in a wash bottle. A rapid air stream was bubbled through it for 48 hours during 6 days. The permanganate required by 10 cc. was determined at the beginning and at the end of the experiment and showed that no perceptible oxidation had taken place. This shows that disodium phosphate does not act like alkali in this respect, but rather conforms to the rôle of a true peroxidase.<sup>19</sup>

#### SUMMARY.

1. The work of Löb on the accelerative effect of phosphate mixtures on the oxidation of glucose with hydrogen peroxide was repeated and confirmed.

2. The confirmation consisted in proving by an adequate method that the destruction of glucose, conceded by all in this case, is oxidation.

3. It was shown that glucose may be quantitatively oxidized to CO<sub>2</sub> with hydrogen peroxide in the presence of the phosphate mixture. This fact it appears was not suspected by Löb, and increases the importance of his observations considerably.

4. The results as a whole show that although optimal OH ion concentration is possibly necessary it is less important than

<sup>19</sup> Cf. Bach, A., in Oppenheimer, C., *Handbuch der Biochemie des Menschen und der Tiere*, Jena, 1st edition, 1913, suppl., 160.

Inorganic compounds known to play the rôle of peroxidase in *in vitro* oxidations have usually been, as Bach states, metallic salts of the heavy metals such as iron and manganese. The synthetic peroxidases of Trillat (Trillat, M. A., *Compt. rend. Acad.*, 1904, cxxxviii, 274), of Dony-Hénault (Dony-Hénault, O., *Bull. acad. roy. belg.*, 1908, 105), etc., prepared from manganese were prepared to resemble and imitate what it was thought are the essential properties of an oxidizing enzyme. The peroxidase disodium phosphate differs from these inorganic peroxidases in that the peroxidase property is dependent on the phosphate part of the molecule. Other sodium compounds do not exhibit the same effect. On the other hand dipotassium phosphate, as was shown by experiments not yet published, has the same effect. That the remaining alkali and alkaline earth dibasic phosphates may act in the same way seems likely.

From this point of view then these results are of considerable interest because we have a compound playing the rôle of peroxidase, in which the non-metallic part of the molecule carries the characteristic property. In this respect it seems likely that it resembles the biological peroxidases more closely than the heavy metal derivative peroxidases do.



Löb's experiments and interpretation would indicate, when the phosphate mixture is used, and that the optimal limits, if they exist, are wider than he states.

In fact it seems more accurate to refrain from emphasizing the segregated OH ion in interpreting the reaction and simply state that the effect is specifically related to the presence of disodium phosphate under suitable conditions.

5. The amount of disodium phosphate used is the most significant factor in determining the reaction. Little or much monosodium phosphate was used with a constant amount of disodium phosphate without producing a marked negative effect on the reaction.

6. The phosphate mixture may be used repeatedly at 37° for the oxidation of additional amounts of glucose owing to the fact that the product (CO<sub>2</sub>) is evolved from the reaction mixture during the process of oxidation. Disodium phosphate accordingly plays the rôle of a typical catalyst.

7. Consequently disodium phosphate functioning in the manner described in this paper is the only chemical substance known to be generally necessary to the life of organisms, that is known to catalyze the quantitative oxidation of glucose to carbon dioxide.

8. That compounds like hexose phosphate ester are the intermediates involved in the acceleration of oxidation described in this paper seems almost certain at first, in the light of the results of Harden and Young,<sup>10</sup> of von Lebedew,<sup>16</sup> and of Embden, Griesbach, and Laquer.<sup>16</sup> The attempts so far made to establish the formation of such a compound failed to demonstrate its formation under these conditions.

9. On the other hand a close parallelism between the rate of spontaneous decomposition of peroxide and the rate of glucose oxidation in the same solutions was established. This together with other facts developed gives experimental basis for the idea that the oxidation really depends upon the intermediate formation of a highly reactive perphosphate.

10. In producing this accelerating effect upon glucose oxidation disodium phosphate does not play the rôle of both oxygenase and peroxidase, as some inorganic compounds do, but acts only as a peroxidase. It is unable to activate atmospheric oxygen to any appreciable extent.

11. That the phosphate catalysis does not depend alone on unlimited capacity to decompose peroxide is clearly shown by the fact that the hydroxide and carbonates of sodium, which are much more effective in decomposing peroxide, diminish the glucose oxidation roughly in proportion to their increased ability to decompose peroxide.

12. Glucose is not oxidized by hydrogen peroxide in solutions containing  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  when these are used in the same molecular concentrations as the two phosphates in the phosphate mixture.

13. These results together with those with sodium hydroxide show that available alkali, contrary to what was observed in the permanganate oxidation of glucose,<sup>4, 20</sup> is without appreciable influence on the oxidation of glucose with hydrogen peroxide.

<sup>20</sup> Witzemann E. J., *J. Am. Chem. Soc.*, 1917, xxxix, 2657.







NUTRITIONAL EDEMA AND  
“WAR DROPSY”

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## NUTRITIONAL EDEMA AND "WAR DROPSY" \*

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Before the recent war, medical literature contained frequent references to the type of edema now recognized as "war edema." With the clinical picture in mind presented by meager reports that have appeared in the American<sup>1</sup> and British scientific journals of recent publication, a somewhat extended study of wars, famines and epidemics of the past has proved fruitful in bringing to light evidence of the prevalence of edema of this type under varying conditions of insufficient and inadequate food. This edema resembles that of renal disease. In mild cases it may be confined to the lower limbs, but in the severe type the edema may extend to all parts of the body. There is no albuminuria. Accompanying this edema there are emaciation, muscular weakness, depression, anemia, and very frequently gastro-intestinal disturbances.

While the term war edema is not found in early medical literature, there is much evidence that the condition known by this term has been of frequent occurrence in the past. In giving a name to this disease, the authors usually express the chief etiologic factor with the most pronounced clinical symptom, so that war edema, prison dropsy, hunger swelling, epidemic dropsy, edema from inadequate food, deficiency edema, edema without albuminuria, and many similar terms have been used. In civil practice previous to the war, essential idiopathic or primary edema, salt edema in children, alimentary dropsy, anemic dropsy and

\* From the Otto S. A. Sprague Memorial Institute.

1. War Edema, Current Comment, J. A. M. A. **70**: 627 (March 7) 1918. Warthin, A. S.: War Edema, Int. A. M. Museums Bull. **7**: 196 (May) 1918.

edema following gastro-enteritis were some of the more common terms employed.

Prinzing<sup>2</sup> makes no mention of war edema from the time of the Peloponnesian Wars, from 430 to 425 B.C., to the siege of Port Arthur in 1904. In a large number of these etiologically related conditions, edema appears as a symptom rather than as a specific disease. In the destruction of the French army before Naples in 1528,<sup>3</sup> "those soldiers who were not confined to bed in their tents were seen with pallid visages, swollen legs, and bloated bellies, scarcely able to crawl."

Vacher,<sup>4</sup> writing of the conditions of childhood during the siege of Paris in 1870-1871, finds that the effect of insufficient nourishment showed itself in progressive emaciation, edema of the integument, anemia, and uncontrolled diarrhea, which were characteristic symptoms of the hunger fever which decimated the infant population. Between 1877 and 1880, there broke out in Calcutta a peculiar disease to which the term "epidemic dropsy" was applied.<sup>5</sup> This disease followed an extensive famine in southern India.<sup>6</sup> It persisted in epidemic form until 1880. The number of cases increases in each cold season and falls off in each hot season. It has continued to appear in Calcutta sporadically. Neighborhood and family groups continued to be reported until 1915.

Edema of war occurred during the Napoleonic campaigns, the siege of Paris, and in the concentration camps during the Boer War, when it was known as epidemic edema.<sup>7</sup> Falta<sup>8</sup> of Vienna mentions that the disease was known in Russia during famines before the war, and that the expression "swollen from hunger" was current in the affected districts. Landa<sup>9</sup> related that in August, 1915, when the City of Mexico had been the seat of military operations for two or three months, the appearance of numerous cases of edema

2. Prinzing, F.: *Epidemics Resulting from Wars*, New York, Oxford University Press, 1916.

3. Hecker, J. F. C.: *Epidemics of the Middle Ages*, London, 1846, p. 231.

4. Vacher: *La mortalité à Paris en 1870*, *Gaz. méd. de Paris*, 1871, p. 9, cited by Prinzing (Footnote 2).

5. Green: *Epidemic Dropsy* in *Encyclopedia of Medicine and Surgery*, Edinburgh and London 2: 422.

6. Leys, J. F.: *Epidemic Dropsy*, *Reference Handbook of Medical Sciences*, Ed. 3, New York, William Wood & Co., 3: 696, 1914.

7. Maliwa, E.: *Wien. klin. Wchnschr.* 30: 1477, 1917.

8. Falta, W.: *Wien. klin. Wchnschr.* 30: 1736, 1917.

9. Landa, E.: *Deficiency Edema*, *Gaceta méd., Mexico* 11: 67 (Jan.-June) 1917; abstr. *J. A. M. A.* 78: 424 (Feb. 9) 1918.



began to be noted. Starvation edema was reported by Dr. Patterson<sup>10</sup> in 1899 after a season of famine in China. Holst,<sup>11</sup> in connection with his researches in ship beriberi and scurvy, furnishes this interesting group of historical data:

Many dropsical cases were observed during the Crimean War when scurvy was prevalent. Dropsy without sore gums occurs every year on board the French fishing vessels off the coast of Newfoundland. During the first part of the nineteenth century dropsy was common in European and American prisons. This prison dropsy is stated in 1847 to have been, besides typhoid fever and consumption, the most prevalent cause of death in forty-one prisons in England, France and North America. In 1857, it caused one-half the deaths in a prison in Breslau.

#### RECENT REPORTS OF EDEMA IN EUROPE

The first record of war edema in the recent war was made in 1915 by Strauss, who described the "hunger disease" in Russian Poland and Galicia, where the poor had an insufficient and monotonous dietary, and were exposed to war epidemics. About the same time, in July, 1915, Budzynski and Chelchowski<sup>12</sup> described a series of 110 cases of a peculiar affection occurring among the inhabitants of certain towns in Poland as a result of insufficient and inadequate food caused by the German occupation. The name "hunger swelling" was applied to this disease because its most characteristic feature was the presence of marked edema, recalling that encountered in dropsical beriberi. All the patients suffering from the disease were in a state of semistarvation.

In Germany, war edema first made its appearance in prison camps in July, 1915. Rumper at first considered relapsing fever responsible, but in 1916, when many cases with dysenteric symptoms occurred in prison camps free from relapsing fever Rumper and Knack<sup>13</sup> regarded dysentery as a predisposing cause. At this time, cases were reported among Russian soldiers at the front. Early in 1917, there were many cases in

10. Patterson, A. H.: Starvation Edema, *Med. Rec.*, November, 1899, p. 715.

11. Holst, A.: Experimental Studies Relating to Ship Beriberi and Scurvy, *J. Hyg.* **7**: 621, 1907. Holst, A., and Frölich, T.: *J. Hyg.* **7**: 670, 1907.

12. Budzynski, B., and Chelchowski, J. M. H.: Hunger Swelling in Poland, *J. Trop. M.* **19**: 141 (June 15) 1916.

13. Rumper and Knack: *Brit. M. J.* **2**: 560 (Oct. 27) 1917.

the civil population and labor battalions in Germany, and in the spring, cases appeared in Austria among civilians, especially workmen, but rarely among the troops. The disease appeared in Vienna with great suddenness. In 1917, marked attention was given in German medical journals to this peculiar disease, which seemed to have become widespread throughout Germany. The first cases noted in Berlin were in January, 1917.

Guillermin and Guyot<sup>14</sup> made personal observations and reported in March, 1919, that insufficient food in Russia, Germany and Austria had caused a specific disease of hunger which was known as hunger edema. These authors described the disease as it occurred among French prisoners of war.

In Poland, the patients were in a state of semistarvation, none having eaten meat for several months and some not since the beginning of the war. The cases occurred almost exclusively among the poorest of the people and the unemployed factory hands, who were without money to buy food at the famine prices. The staple diet of the people consisted of potatoes supplemented by small quantities of soup and bad bread on certain days. The amount of potatoes eaten averaged 5 pounds for each person daily, a dietary which caused diarrhea and eventually led to most of the food's being passed through the gastro-intestinal tract undigested. The causes of the disease, according to the Polish authors Budzynski and Chelchowski, were lack of proper food, especially the absence of fats, and the large amount of bad potatoes consumed. Maase and Zondek,<sup>15</sup> in agreement with other authors, consider the cause of the disease to be underfeeding, resulting especially from the diminished quantity of fats. Another factor suggested was the amount of fluid ingested. Owing to the changed conditions, most of the sufferers had been taking a more watery diet than normally, in the form of soups, turnips, etc., and the occurrence of diarrhea was fairly common. Schiff<sup>16</sup> suggests that this purely war disease has obvious similarities to beriberi and other diseases resulting from

14. Guillermin, R., and Guyot, F.: Undernourishment and Famine Edema, *Rev. méd. de la Suisse Rom.* **39**: 115 (March) 1919.

15. Maase, C., and Zondek, H.: *Berl. klin. Wehnschr.* No. 36, Sept. 3, 1917; abstr: *Brit. M. J.* **2**: 560 (Oct 27) 1917.

16. Schiff: *München. med. Wehnschr.* No. 22, 1917.

a lack of vitamins. Falta says that all of the patients had been improperly fed for a long time, especially as regards proteins, and the liability to edema, always present in malnutrition, was aggravated by the large quantity of sodium chlorid in the food. He states that persons showing war dropsy had usually been getting from 1,200 to 1,400 calories a day, including only 30 to 50 gm. of protein. But as such degrees of edema do not ordinarily occur in simple starvation, he believes that another factor must be present in these cases, namely, the ingestion of large amounts of fluid and salt in the attempt to sustain life on the thin vegetable soups common in prison camps and in famine districts. Cold, hard work and infectious diseases increase the tendency to edema simply because they increase the deficiency in food calories.

The Swiss authors found that in some regions the conditions of underfeeding were extreme, resembling the great famines of history. The disease was also very frequently found in men who were subject to hard work on a diet of from 800 to 1,200 calories, consisting of 15 per cent. and more of indigestible cellulose, bread containing 97 per cent. potatoes, very little fat, and a ration of 50 gm. of albumin, daily, at the highest. Exposure to cold and hard physical labor were contributing factors in the development of this disease.

Maase and Zondek<sup>15</sup> suggest that the toxic products of protein metabolism may cause damage to the endothelial lining of the blood vessels and so lead to edema. The high residual nitrogen and ammonia values found by them in the urine, blood and edema fluids were considered to be evidence of this hypothesis. Franke and Gottesmann,<sup>17</sup> in their study of the functional efficiency of the kidney in seventeen cases of war edema, found delay in excretion of urea and sodium chlorid in ten patients, and of potassium iodid and lactose in seven. They therefore call war edema a nephritis without albuminuria.

Maliwa,<sup>7</sup> from investigations of four cases, correlates the stage of polyuria with an excess of sodium chlorid in the blood, and finds that after the polyuria has passed off the blood is deficient in the sodium chlo-

17. Franke, M., and Gottesmann, A.: *Wien. klin. Wchnschr.* **30**: 1004, 1917.

rid content. The change in the osmotic relations of the tissue is the essential factor in the disease, the polyuria and edema, though prominent clinical features, being secondary in importance. To determine the cause of the edema, Knack and Neumann<sup>18</sup> sought to secure its return in convalescents by restricting the diet principally to turnips, and by giving large quantities of water internally. Neither measure separately ever produced the result, but with the restriction of the diet, plus water drinking, the edema rapidly returned in convalescent patients. Lange<sup>19</sup> discusses the causation of a group of cases observed by him in West Prussia, and concludes that an altered permeability of the blood vessels was present, owing to a qualitative alteration in the food, perhaps to the extreme deficiency of calcium. Hulse<sup>20</sup> reaches similar conclusions, except that the edema was more frequently found as a sequel to relapsing fever, and in men who had been previously exposed to extreme cold. Recent writers discuss the subject of vitamins and the rôle they play in deficiency diseases, especially those belonging to the beriberic type, but generally recognize that it is not a well-defined deficiency disease, and in the majority of cases is the result of an inadequacy of diet to supply the nutritional requirements of the body.

The characteristic symptoms found among the inhabitants of certain towns in Poland were edema, debility, muscular weakness, intestinal disorders, mental depression, dimness of vision, disappearance of sexual impulses, and alterations in the blood and urine. With the disappearance of the edema the wasting was evident, the patients sometimes being reduced to mere skin and bones. Maase and Zondek<sup>15</sup> state that there were no noteworthy premonitory symptoms, but suddenly marked edema developed, especially in the lower limbs, with anemia and frequent diarrhea. Among French prisoners, there was great emaciation, the loss of weight being frequently 40 per cent. of the initial weight, anemia, general edema, muscular weakness and nervous exhaustion: and apathy and depression were

18. Knack, A. V., and Neumann, J.: Outbreaks of Edema in Germany. *Deutsch. med. Wchnschr.*, July 19, 1917, p. 901; abstr. *Lancet* **2**: 248 (Aug. 18) 1917.

19. Lange, F.: *Deutsch. med. Wchnschr.*, July 12, 1917, p. 876; abstr. *Lancet* **2**: 248 (Aug. 18) 1917.

20. Hulse W.: *München. med. Wchnschr.*, July 10, 1917, p. 921; abstr. *Lancet* **2**: 248 (Aug. 18) 1917.



associated with a "facies pestica." Falta states that prostration, apathy and weakness were almost constant; a feeling of heaviness in the legs, and diminished reflexes were found, but no typical polyneuritic symptoms occurred. The nutritional value of the food was still further diminished by diarrhea and dysentery, which were frequently associated early in the development of the disease.

In cases among the civil population, the edema was generally located in the feet and legs, occasionally in the thighs and trunk. In more than one-half the cases there was some degree of facial swelling; in one-sixth, the hands were swollen, and in one-ninth ascites was present. The face and scrotum were often affected, and in a small number of cases, ascites and hydrothorax occurred. Its features were remarkably uniform: the edema resembled that of renal disease and in mild cases was confined to the lower limbs; but in severe cases it was universal and caused considerable limitation of movement, sometimes interfering with the opening of the eyes. The edematous tissue was soft and elastic; the skin and puncture fluids were pale. In some cases the edema came on gradually, but after severe physical exertion, more rapidly. Following the disappearance of the dropsy, relapses were prone to occur, especially if there was any return to hard work or unsuitable food. The edema sometimes led to bursting of the skin with serous exudation, or so stretched the skin that pink scars like striae gravidarum resulted from it. The swollen extremities felt cold, and were painful when touched. Beyermann<sup>21</sup> states that twelve cases among the insane suggested scurvy or purpura except for the remarkably slow pulse and the absence of changes in the gums. On the addition of fresh vegetables to the ordinary diet, conditions returned to normal.

The urine was usually pale like water, alkaline, and contained neither sugar nor albumin. The amount of urine passed varied greatly in different cases, but on the whole was increased, sometimes reaching 60 ounces and over when the swelling was disappearing. As

21. Beyermann, W.: Edema Disease in the Netherlands, *Nederlandsch Tijdschr. v. Geneesk.* **1**: 2265 (June 28) 1919; abstr. *J. A. M. A.* **73**: 1172 (Oct. 11) 1919.

soon as the patient was put to bed, a marked diuresis began, and during the stage of recovery the amount of urine passed daily was from 3 to 4 liters. High residual nitrogen and ammonium values were found in the urine, blood and body fluids. Falta found polyuria and frequency of micturition; the urine being clear, of low specific gravity, and free from albumin and formed elements, except a few hyaline casts. Tonin<sup>22</sup> comments on the odd fact that polyuria constantly accompanied the hunger edema in ex-prisoners of war seen at the hospital. As noted by others, this polyuria usually began when the patients were at rest in bed.

Jensen's<sup>23</sup> study of the blood showed from 1.5 to 4 million red corpuscles with a color index greater than one, usually with a leukopenia, in 60 per cent. of the cases there being less than 5,000, with a relative lymphocytosis (from 30 to 55 per cent.). The coagulation time was usually shortened, and the blood proteins were nearly always decreased, generally being from 4 to 6.4 per cent. (normal is from 6.5 to 8.5 per cent.), that is, there was a hydremia with hypo-albuminosis. The freezing point was normal, the residual nitrogen normal or low, uric acid normal, and sugar and calcium usually low, chlorin usually approaching the upper normal figures, although it was occasionally low. Chemical examination of the blood and urine (Knack and Neumann) revealed a diminution in lipoids and in the organic phosphorus content of the blood. The depletion of the tissues in nutritive reserve in war dropsy is shown by Falta's statement that when absolute fasting is studied in these cases there are only from 2 to 3 gm. of nitrogen eliminated per day, as against 10 to 12 gm. of nitrogen excretion during the fasting of normal persons.

There were no cardiac symptoms reported by Maase and Zondek, but other observers found a condition suggestive of a cardiac lesion with failing compensation. Falta states that the slow pulse, from 35 to 40 a minute, characteristic of war edema, is best marked in males. Schiff reports a somewhat higher pulse rate of from 42 to 56. The edema was frequently observed with cardiac symptoms and infections in children, but in adults without these complications.

22. Tonin, R.: *Gazz. d. osp.* **40**: 636, 1919.

23. Jensen: *München. med. Wehnschr.* **65**: 925, 1918.

Hemeralopia frequently preceded the development of the edema. In severe cases, corneal ulcer and xerosis of the conjunctivae were troublesome. Ophthalmologists describe these eye changes as the result of debility and poor nourishment. Nyctalopia, or night blindness, is common in the spring and fall as a symptom of debility. Night blindness seldom occurs as a functional disorder except in cases of general debility, starvation or scurvy. The development of xerophthalmia is now recognized as due to a specific deficiency in fat-soluble vitamins. Maynard<sup>24</sup> discussed twenty cases of increased intra-ocular tension found in the course of epidemic dropsy. There was dimness of vision, the cornea was a little steamy, and the pupils were small or moderately dilated. The tension of the eyeball was distinctly increased. Halos, generally rainbow-like, were complained of at one time or another during the attack of dropsy.

Vandervelde and Cantineau<sup>25</sup> made observations on 200 patients treated by them in the St. Pierre Hospital at Brussels. Most of these cases were among deported Flemish civilians. There was marked edema of the lower limbs, frequently associated with "grave phlegmons." The general condition was brought about by lack of food and by deplorable hygiene. There were weakness and profound anemia; and dyspnea resulted from the slightest effort. Those deported were recruited without any medical examination and were forced to do hard physical work. Minor symptoms and complications were common. Among these were: ringing in the ears and dry, painful skin with frequent secondary pyogenic infections; and in one or two instances dark pigmented patches were observed on the face, similar to the pigmentation in Addison's disease. (Noted by the Polish authors.)

In mild cases under the influence of a more generous dietary, recovery took place. The regulation treatment for the condition consisted in a better diet as far as possible and rest in bed until all swelling had disappeared. Knack and Neumann found that recovery always followed rest in bed on ordinary hospital diet

24. Maynard, F. P.: Preliminary Note on Increased Intra Ocular Tension Met with in Cases of Epidemic Dropsy, *Indian. M. Gaz.* **44**: 373, 1909.

25. Vandervelde, M., and Cantineau, M.: Edema Among the Deported, *abstr. J. A. M. A.* **73**: 1229 (Oct. 18) 1919.

and that the restriction of fluids was rarely necessary. Maase and Zondek, by giving three patients 100 gm. of fat daily for a week, were able to cure the disease completely without rest in bed or other remedial measures. The diet should be ample, especially in regard to protein. The lack of resistance to cold is striking, death following relatively slight chilling, so that warmth is an important part of the treatment. The prognosis is good if the patients are kept in bed on a proper diet, but severe cases frequently prove fatal. Postmortem findings are seldom reported. Chronic marasmus with atrophy of the viscera, especially the heart and spleen, fatty degeneration of the liver and kidneys, and in some instances, dysenteric ulcers were found. In three cases Budzynski and Chelchowski found a diminution in the amount of blood, and a reduction in the size of the liver.

#### REPORTS FROM INDIA, CHINA AND MEXICO

Leaving the recent reports of edema in Europe and turning to the literature of other countries, we find that in many lands similar epidemics of dropsy have resulted from famine. Until the appearance of "epidemic dropsy" in India following the famine in 1876-1877, "swellings" were regarded as a minor symptom, when arising in the course of famine diseases. During this famine the mortality was high, and in eight famine districts nine tenths of the total recorded deaths were caused by famine diseases—dysentery, dropsy, diarrhea and debility.<sup>26</sup> Government works and a system of rationing were established for men, women and children unable to earn the daily ration. To test the value of this ration, a system of weighing the people was undertaken. In these tests great caution was found necessary for, it was reported, many of the people who came into the camps appeared to be filling out and fattening, when in reality they were getting dropsical and in a fair way to die. In the nursery of the famine relief camp near Madras, many children were found to be in a dropsical condition, and most of the old people were in the same state. Old men and old women were bloated with dropsy, and others again, many of them

26. Digby, W.: The Famine Campaign in Southern India, 1876-1877.



in the prime of life, were mere skeletons, the bodies of full grown men weighing only from 58 to 85 pounds at necropsy.

To supply the vast population of southern India with the necessary amount of food for health was the "Hoover problem" of the famine relief agencies. Practically all the grain had to be imported, and transportation facilities were inadequate. It seemed necessary to keep the grain ration, principally rice, as low as possible. Animal foods were scarce. Dr. Cornish, adviser of the government of India on public health questions, pointed out that effects of insufficient nourishment might not be immediately apparent, and throughout the famine constantly emphasized the importance of the nitrogenous value of the ration, and advocated a ration consistent with age and work, sufficient to replace tissue waste. After this famine, reports began to appear in the *Indian Medical Gazette* of acute dropsy and acute anemic dropsy. In 1881, McLeod<sup>27</sup> termed the disease "epidemic dropsy." The "new disease" continued to be the subject of many reports and extensive bacteriologic investigations until 1909-1910. According to bacteriologic phraseology, it appeared endemically and epidemically, and much study was given to a specific organism, with no constant results.

Dr. Greig,<sup>28</sup> in his report on epidemic dropsy, states that there is evidence to show that epidemic dropsy is a nutritional disease which is brought about by a one-sided dietary, and that the two severe outbreaks of epidemic dropsy in Calcutta and Bengal, namely, from 1877 to 1879, and from 1907 to 1909, have been correlated with a sustained high price of food grains during this period, and the cessation of these epidemics has synchronized with the fall in prices of food grains. The study of the parasitic origin of disease has somewhat overshadowed the question of the relation of defects of dietary to the causation of disease in the tropics. In one locality, Greig found in 321 houses, with 4,637 inhabitants, 1,581 persons who were dropsical. The persons attacked consumed polished rice, and this was their staple diet. The amount of rice

27. McLeod, K.: Epidemic Dropsy in Calcutta, *Indian M. Gaz.* **16**: 148, 1881.

28. Greig, E. D. W.: The Scientific Memoirs of the Government of India, No. 49, 1911-1912.

consumed daily varied from 2 to 16 ounces (from 1 to 8 chittaks). When the price of grains rose, the capacity for purchasing additional suitable articles of diet diminished and the diet became dangerously onesided.

The peculiar qualities of rice as a diet were pointed out by McCay<sup>29</sup> in his investigations of jail dietaries. Rice is the poorest of all cereals in protein, and when cooked it swells up and absorbs three and one-half times its weight in water. The percentage of starch in rice is high—up to 80 per cent. Rice is deficient in fat. Rice is a bulky diet, 26 ounces of dry rice when cooked measuring about 2,800 c.c. A large carbohydrate diet, by its mere presence in the intestinal canal, hinders the absorption of protein. On account of the fermentation processes that are quickly set up, there is increased peristalsis, and the food is hurried through the small intestine past the area most favorable for absorption. The amount of rice present in the diet influenced in a marked degree the quantity of urine excreted. The rice may have a diuretic action on the kidneys, or water may be formed in the tissues from the constituents of the rice, in addition to the large water intake with the boiled rice itself.

Dr. Patterson<sup>10</sup> of Chinkiang, China, described a group of cases of dropsy occurring in dispensary patients after a famine season. The food of these people consisted largely of weeds and wild plant greens. As no literature could be found on the subject, the disease was called "greens dropsy." The only symptom complained of was the swelling. With some medical treatment and money to buy grain, the patients recovered rapidly.

When the City of Mexico had been the seat of military operations for two or three months, Landa<sup>9</sup> related that many cases of edema in men, women and children began to be noted. Hundreds of cases were found with no albuminuria. As in other famine epidemics, many persons died of actual starvation, while others developed edema cachexia from defective nourishment. The mortality was high, the patients dying in marasmus with heart failure. There was hydremic anemia, hypothermia, slow pulse, reduction

<sup>29</sup> McCay, D.: *The Scientific Memoirs of the Government of India*, 1909-1911.

of the reflexes, and pain in the muscles. The only food obtainable had been vegetables of the families Chenopodiaceae and Amaranthaceae, such as beets and spinach.

#### RELATION OF WAR EDEMA TO DEFICIENCY DISEASES

Frequent reference is made to the similarity between the clinical symptoms found in war edema and those associated with diseases of the beriberi type. Falta states that the wet form of beriberi is the only other deficiency disease in any way resembling war edema. In this group of edematous diseases, as discussed by various authors, are tropical beriberi, ship beriberi and epidemic edema. The polyneuritic symptoms in tropical beriberi have been so constantly emphasized that they have obscured the equally important edematous conditions which form the chief feature in the wet type of the disease. In epidemic edema and ship beriberi, nervous phenomena are rarely present, but edemas of various degrees constitute the major symptom. Infants nursed by beriberic mothers suffer from edema, dyspnea and cyanosis. Authorities agree that this is an infantile beriberi due to some deficiency in the mother's milk. Almost all cases of infantile beriberi are edematous. The pathologic findings observed at necropsy in 219 infants under 1 year of age showed a percentage of 56.6 of infantile beriberi. Vedder and Williams<sup>30</sup> regard this edema in infantile beriberi as due to a specific avitaminosis. Vedder<sup>31</sup> furnished a list of food deficiencies found by the various investigators in beriberi: (1) deficiency in fat (Bremaud and Laurent); (2) nitrogen starvation (Takaki); (3) deficient vegetables combined with an infection (Fales); (4) deficiency in organic phosphorus (Schauman) and (5) deficiency of some unknown substance, not phosphorus (Fraser and Stanton, Chamberlain and Vedder, Shiga and Funk).

It is interesting to contrast with this group the findings by the various authors in war edema. The lack of calcium, fat, phosphorus in the blood, fresh vegetables, proteins and vitamins have each been emphasized in war edema. In addition there was general

30. Vedder, E. B., and Williams, B.: Concerning the Beriberi-Preventing Substances or Vitamines Contained in Rice Polishings, *Philippine J. Sc.*, Sec. B, **8**: 175, 1913.

31. Vedder, E. B.: *Beriberi*, New York, William Wood & Co., 1913.

underfeeding; the diet as a whole was low in caloric value. The food was quantitatively as well as qualitatively deficient. There was semistarvation.

Lind,<sup>32</sup> in his early account of scurvies, found dropsy a constantly recurring symptom. Scorbutic persons were found to have edematous swellings at first about the ankles, later extending to the legs and other parts. The face, especially, became pale, swelled and bloated. Long want, improper diet, melancholy and cold are given among the causes. Dr. Cook, in a letter to Lind at this time, finds the term "nervous disorders" universally applied to most chronic and cachetic ailments. The lower people "who live continually on farines and a gross diet," and among whom these complaints are found, had a universal lassitude, pains which they termed rheumatic, and a breathlessness on exercise. The legs were sometimes swollen and the abdomen almost always tender and tumefied. Professor d'Espine observed these edemas during the siege of Paris as a first stage of scurvy; and Guillermin and Guyot, commenting on similar scorbutic complications, ask if scurvy may not be simply a state more advanced in the evolution of this disease, of which edema is an initial symptom. But the number of deaths occurring without scorbutic symptoms seems to plead for war edema as "*une entité morbide.*" Dropsical patients without sore gums were frequently observed in epidemics of scurvy in Russia during the Crimean War when scurvy was very prevalent.

In pernicious anemia associated with pregnancy, Williams<sup>33</sup> finds anemia, weakness, shortness of breath, and edema of the extremities. A general puffiness affecting the hands and face as well as the legs, without urinary findings, is common in hydremic patients. More than half of all pregnant women, according to DeLee,<sup>34</sup> show some edema of the feet, the hands or the face. Often this is an elastic puffiness that does not pit. The cause of this is not known. In reproductive processes throughout nature, growth occurs at the expense of the maternal tissue. The protein materials are chiefly concerned in the growth of the

32. Lind, J.: *A Treatise of the Scurvy*, Edinburgh, 1753, p. 319.

33. Williams, J. W.: *Obstetrics*, New York, D. Appleton & Co., 1912, p. 509.

34. DeLee, J. B.: *The Principles and Practice of Obstetrics*, Ed. 2, Philadelphia, W. B. Saunders Company, 1915, p. 386.



new cells. Miescher<sup>35</sup> showed that salmon, after entering the Rhine from the sea, virtually starve. Yet the genital organs of both male and female develop greatly at the expense of the liquefying muscles, which may lose 55 per cent. of their weight (protein) without destruction of the muscle cell.

In war edema and in the etiologically related edemas in deficiency diseases, hydremic anemia is a somewhat frequent symptom. Osler and McCrae,<sup>36</sup> in their study of the circulatory disturbances in a group of cases of chlorosis, find dyspnea in 318, palpitation in 254, and edema in 231. "Doubtless it is the occurrence of slight degrees of edema which gives chlorotic patients so plump a look." All the symptoms come on in the course of from three to twelve months. The disease is most common in ill fed and overworked girls.<sup>37</sup> A long continued unbalanced diet may play a large part in the process.

Sir Joseph Fayrer<sup>38</sup> finds that pernicious anemia in Europe resembles beriberi in the Orient. Bramwell,<sup>39</sup> in a table showing the most important symptoms in forty-five cases of pernicious anemia, records twenty-three cases of dropsy, associated with great prostration, weakness and loss of weight. The urine was normal in the majority of cases. This edema was considered as partly due to the watery condition of the blood, and partly to the enfeebled state of the heart. Functional derangements of the stomach and intestine are almost invariably present. A symptom<sup>40</sup> which is practically never wanting is edema, especially of the legs and under eyelids, though it is also seen in other places on the body. The swelling is practically never marked, but is very persistent, and is noticeable as one of the earliest symptoms of the disease. Moreover, it readily recurs in patients who show a complete remission. As in other anemias, the edema is possibly due to alterations in the blood vessel walls. A gain in body weight

35. Miescher, quoted by Lusk, Graham: *The Science of Nutrition*, Ed. 2, Philadelphia, W. B. Saunders Company, 1909.

36. Osler, William, and McCrae, Thomas: *Modern Medicine*, Ed. 1, Philadelphia, Lea & Febiger, 1915.

37. Osler, William: *Principles and Practice of Medicine*, Ed. 8, New York, D. Appleton & Co., 1916, p. 730.

38. Fayrer, Joseph: *Beriberi*, in *Quain's Dictionary of Medicine*, London, 1888, p. 104.

39. Bramwell, Byron: *Anaemia*, Philadelphia, William Wood & Co., 1899.

40. Stengel, Alfred: *Diseases of the Blood*, Philadelphia, W. B. Saunders, 1905, p. 263.

in pernicious anemia when unattended with increase of hemoglobin indicates dilution of the blood and escape of serum into the tissues.

Edema occurring in the course of gastro-intestinal disorders and marasmic conditions in infancy is somewhat infrequent but well recognized by pediatricians. Chapin<sup>41</sup> reports twenty-one cases of general and local edema in which neither the condition of the blood nor that of the urine explains satisfactorily the development of the edema. The clinical conditions in which these edemas are most frequently found are: (1) difficult digestion and malassimilation with gastro-intestinal disturbances and diarrhea; (2) exhaustive conditions, such as prematurity, marasmus, extreme secondary anemias, edema neonatorum, and in long debilitating diseases; (3) occasionally in various constitutional diseases, such as syphilis, tuberculosis, erysipelas, and pertussis, and (4) in angioneurosis of vasomotor origin.

Under the term essential, primary or idiopathic edema, Wagner,<sup>42</sup> in 1887, records the earliest account of this disease. An epidemic of edema in which thirteen cases occurred in thirty-five babies in which gastro-enteritis was prevalent was thought by De Wolf<sup>43</sup> to be of infectious origin. The cases all occurred within a short time in a children's hospital in which the food supply was modified milk alone, or modified milk with the addition of a cereal or a proprietary food.

Potter,<sup>44</sup> in a group of cases of diarrhea with edema following a diet of barley water with a low percentage of fat and protein, increased the fats and proteins with the disappearance of the edema in a short time. The same author later reports a large group of cases in which he considers the edema a symptom of malnutrition and marasmus. In typical cases these babies had been treated for some time with boiled water, barley water or whey. A slight gain in weight occurred as the edema developed. Potter says that it is not what

41. Chapin, H. D.: Cases of Edema in Infants, *Arch. Pediat.* **31**: 5, 1914.

42. Wagner, E.: *Deutsch. Arch. f. klin. Med.* **41**: 509, 1887.

43. DeWolf, H.: A Report of Thirteen Cases of Edema Apparently Epidemic in Character, *Arch. Pediat.* **19**: 895, 1902.

44. Potter, P. A.: The Relation of Protein to Edema in Marantic Children, *Med. News*, New York, Jan. 9, 1904; Edema in Infants, *Arch. Pediat.* **29**: 206, 1912.

the babies are being fed that causes the dropsy, but what they are not being fed; also that it is entirely owing to the fact that they are not getting enough proteins in the diet, and this notwithstanding the intestinal disturbances that practically always accompany or precede the edema. It may be that in many of the cases the continuance of the diarrhea itself is due to the deprivations of solids in the food.

Czerny and Keller<sup>45</sup> use the term "Mehlnährschäden" to describe a condition found in infants fed on a high carbohydrate diet, but lacking in other important foodstuffs. The tendency of the tissues to hold water is increased in carbohydrate feeding. Holt<sup>46</sup> finds general edema as a symptom in marasmic infants. There is often increase in weight, and the whole body may become waterlogged. The symptoms shown by some infants that have been fed for a long time on an almost exclusive carbohydrate diet indicate that they suffer from "Mehlnährschäden." The carbohydrate diet is frequently given in the form of proprietary foods and cereal decoctions to overcome diarrhea. Bloch<sup>47</sup> applies the term carbohydrate dystrophy to a group of cases in which he found xerophthalmia associated with edema resulting from fat deficiency and a carbohydrate diet. Hume<sup>48</sup> observed thirteen cases in which edema appeared following gastro-enteritis and vomiting. There was no marked error in the diet to throw light on the etiology of the condition. His observations on salt retention in these infants failed to be conclusive, and as there was no evidence of kidney or heart disease, the pathologic condition was sought for in the tissues themselves. The action of toxins, developed in the gastro-intestinal tract, on the suprarenals or capillary cells is suggested as a possible cause of the condition.

Ashby<sup>49</sup> finds these edemas following gastro-intestinal catarrh which has persisted for weeks. The gastro-intestinal tract is so deranged that poisons absorbed from it reach the systemic circulation and in this way lower the vitality of the endothelium of the

45. Czerny and Keller: *Des Kindes Ernährung*, 1906.

46. Holt, L. E.: *Diseases of Infancy and Childhood*, New York, 1916.

47. Bloch, C. E.: Xerophthalmia and Dystrophy in Infants, *Ugeskr. f. Læger* **80**: 815 (May 23) 1918; abstr. *J. A. M. A.* **71**: 322 (July 27) 1918.

48. Hume, W. E.: General Edema Following Gastro-Enteritis in Children, *Brit. M. J.* **2**: 478 (Sept. 2) 1911.

49. Ashby, H. T.: *Practitioner*, London, May, 1914, p. 686.

blood vessels, causing an increased permeability. Recurrences were common, and these children seemed to do better on food containing a high percentage of proteins with a low percentage of carbohydrates.

In a review of the literature on osmosis and edema in infancy and childhood, Waterman,<sup>50</sup> as late as 1914, finds uncertainty as to the methods of the production of this edema. In the light of present knowledge, the weight of evidence seems to be in favor of the chlorid retention theory of infantile or essential edema, although the vascular lesions theory has many points in its favor. The etiologic factors considered by this author are: (1) latent or hidden nephritis; (2) chlorid retention which leads to a hydremia and so to an edema, and (3) increased permeability of the capillary walls.

In reviewing these various etiologic factors, there is evidence that the same type of dietetic and pathologic conditions is found in these edemas in infants as those concerned with war edema and the edemas found in the deficiency diseases of the beriberi type.

A general dropsy is a common symptom in hydremic animals. Friedberger and Fröhner,<sup>51</sup> and Hutya and Marek<sup>52</sup> describe this condition as it occurs in draft oxen and horses that work in sugar factories and in other cattle from exclusive feeding on distiller's wash. The disease is chiefly caused by feeding on beet root residue, which contains only about 5 per cent. of solid matter with 95 per cent. of water. As the proportion of proteins in the solid matter is only 1 to 10, the residue contains 0.5 per cent. proteins. Consumption of such food combined with hard work results in hydremia. All tissues are infiltrated and the body cavities filled with transudate.

A similar condition of dropsy or "cachexia aquosa" is found in sheep from insufficient pasturage and unfavorable climatic conditions, such as wet or cold weather, badly situated grazing lands, and penning the sheep on wet, cold soil.<sup>53</sup> Weakness, emaciation, anemia, depression and exhaustive diarrheas accompany this condition.

50. Waterman, L.: Arch. Pediat. **31**: 135, 1914.

51. Friedberger, Franz, and Fröhner, Eugen: Veterinary Pathology, Ed. 6, Chicago, W. T. Keener Company **2**, 1908.

52. Hutya, Francis, and Marek, Josef: Pathology and Therapeutics of the Diseases of Domestic Animals, Chicago, Alex. Eger **1**, 1916.

53. Hoare, E. W.: A System of Veterinary Medicine, Chicago, Alex. Eger, **2**: 1290, 1915.



## EXPERIMENTAL EDEMA

Denton and Kohman<sup>54</sup> find that dropsy occurs in a large percentage of rats fed on a carrot diet, when the proportion of nitrogen is reduced by the addition of some non-nitrogenous foodstuff, such as fat or starch. Kohman,<sup>55</sup> in further experimental work, produced edema in a large percentage of rats fed on a diet composed largely of carrots. The addition of fats or fat-soluble vitamin, or water-soluble vitamin, or increase in salt content of the diet had no noticeable effect on the occurrence of edemas, but there was much more marked edema when there was much water in the diet than when the animals were on a dry diet. On the addition of a sufficient amount of an adequate protein to the carrot diet without change in caloric value, no edemas occurred and the animals grew normally. Control experiments showed that the edema was not due to toxic products in the carrots, or to starvation from low caloric intake.

Harden and Zilva<sup>56</sup> observed edema in one of three monkeys fed on a diet complete in every respect, except that it lacked the fat-soluble "A" factor and was low in fat. Each of these animals received a daily diet of from 250 to 300 gm. of boiled, polished rice, marmite, 10 gm., and salt mixture, 2 gm. (The large amount of rice in this diet may have hindered the absorption of the protein.)<sup>29</sup>

Extensive experimental work was conducted by Holst and Frölich<sup>11</sup> in an endeavor to produce ship beriberi in animals. Abortive cases of scurvy resembling ship beriberi were repeatedly seen in guinea-pigs, but although these authors were unable to produce typical ship beriberi they frequently observed edema.

I have carried out a number of dietetic experiments with dogs, rats and guinea-pigs. These animals have been variously fed on specially prepared breads containing much cornstarch in order to reduce the protein content; also, in the case of the rats and guinea-pigs, diets of beets, turnips, cabbage and potatoes with or

54. Denton, M. C., and Kohman, Emma: Feeding Experiments with Raw and Boiled Carrots, *J. Biol. Chem.* **34**: 249 (Nov.) 1918.

55. Kohman, Emma: The Experimental Production of Edema as Related to Protein Deficiency, *Am. J. Physiol.*, to be published.

56. Harden, A., and Zilva, S. S.: Edema Observed in a Monkey Fed on a Diet Free from Fat Soluble "A," Accessory Food Factor and Low in Fat, *Lancet* **2**: 789 (Nov. 1), 1919.

without the addition of starch bread or plain bread. It has not been possible to carry out this work to the extent desired to make a complete study of the subject; furthermore, the work of Miss Kohman seems to cover the ground sufficiently well. Therefore no details of this work will be published. To summarize the results it may be said that in a number of animals edema was obtained, and that these cases occurred under such conditions as to agree fully with Miss Kohman's conclusions. That is to say, edema was not observed in animals that received a dry diet even when they were allowed to take such water as wanted. Most of the instances of distinct edema were observed in animals that lived on a diet poor in protein and fats and containing much fluid. For example, no edema was observed in guinea-pigs living on potato and rye bread, or on meal bread or rye bread alone; whereas a few of the guinea-pigs living solely on beets or cabbage showed more or less edema. A few rats fed solely on a carrot diet also showed edema. In one of these the visible edema disappeared when casein was added to the diet and returned when the animal was again restricted to carrots. This work adds nothing to Miss Kohman's observations, but furnishes merely a certain amount of additional corroboration.

#### GENERAL CONCLUSIONS

It will be seen that the final conclusions reached by those who have studied war dropsy are in extremely close accord. This condition seems not to be a typical "deficiency disease" in the sense of being the result of a deficiency in one or more specific unknown constituents (vitamins) in the diet. In a broader sense it is, however, a deficiency disease, and is the result of a protracted existence on a diet deficient in total calories, especially in protein. Undoubtedly, a high fluid intake, and possibly a high salt intake, are important accessory features. Hard work and exposure to cold are factors simply in that they increase the caloric deficiency of the food supplied.

It is gratifying to find that the experimental work agrees perfectly with the clinical evidence in establishing that a combination of low calories, low protein and excessive fluid intake will lead to a marked dropsy cor-

responding to war dropsy in all respects. The importance of specific vitamins seems to be excluded by these experiments.

Undoubtedly, dropsy occurring in many conditions associated with either defective food supply or absorption (as in some types of infantile dropsy) or in conditions of protracted anemia or cachexia is essentially the same as war dropsy. Hence the general term "nutritional edema" is to be recommended for this class of cases.

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# Elephantiasis Congenita Angiomatosa (Unna)

Associated with Changes in the Capillaries



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AND  
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CHICAGO



# ELEPHANTIASIS CONGENITA ANGIOMATOSA (UNNA)

ASSOCIATED WITH CHANGES IN THE CAPILLARIES \*

MILO K. MILLER, M.D., AND KARL M. NELSON, M.D.  
CHICAGO

## REPORT OF CASE

*History.*—R. T. B., a boy, 6 years of age, was admitted to the hospital, October, 1918, with a very marked dilatation of the veins on the right thigh, leg, scrotum and penis. He is the third of five children, all the others being normal. Nothing particular is to be noted in the family history. The personal history does not contain any other feature of interest, with the exception of some deficiency in talking.

The mother noticed the swelling of the veins on the leg when the patient was 2 weeks old. When 1 year old, rubber bandages were applied, affording some relief. Such bandages have been applied at intervals since, but they were unable to prevent a gradual increase of the dilatation.

*Physical Examination.*—The physical examination revealed no abnormalities besides the condition of the right leg, penis and scrotum. The venous enlargement which forms tumorlike masses, beginning to the right of the scrotum, covers the entire buttocks and extends over practically the entire outer aspect of the thigh and leg, being particularly marked from the knee on down. Greatly dilated veins stand out over the dorsum, the outer aspect of the foot and extend about halfway across the plantar surface.

The swellings felt soft, elastic and velvety; over the foot they were more lobulated and "wormy." The summits of the irregularities were of a bluish color. They were turgescient but not erectile. The blood could be pressed out, leaving an apparently normal delicate skin. The tumor did not pulsate. The definition from the surrounding skin was fairly well defined. In several planes thrombi could be felt. The vascular masses became intensely engorged on standing. There was a mass of dilated deep purplish veins on the scrotum, penis and a small mass of hemorrhoidal veins (Figs. 1 and 2).

MEASUREMENTS OF RIGHT AND LEFT LEG ON STANDING AND IN  
RECUMBENT POSITION

	Right		Left	
	Standing, Cm.	Recumbent, Cm.	Standing, Cm.	Recumbent, Cm.
Upper half thigh.....	32	31	30	30
5 cm. above patella.....	25.5	24	23.5	23
Patella.....	27	26	25	25
Calf.....	22	20	21	20
5 cm. above malleolus.....	15	14	15.6	14
Dorsum foot.....	17.5	16.5	16	16

One small angioma about the size of a pinhead was located on the right nostril.

The blood Wassermann was negative and the eyegrounds were normal.

\* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital.

## COMMENT

Cases as the one described seem to be quite rare. Unna<sup>1</sup> quotes two cases involving chiefly one leg, but he does not give the references. Histologically, the lesions are found in the superficial layers of the corium. They may extend deeply and involve the subcutaneous tissues. The venous capillaries are chiefly involved. Thickening of



Figs. 1 and 2.—Anteroposterior and lateral views showing the angiomatous condition of the right leg and the genitalia.

the walls of the veins is followed by endothelial proliferation. Elastic fibers and muscle fibers are absent.

One of the most striking features of the disease is its apparent strict localization. This made it quite natural that only factors acting locally have been considered in the etiology of these angiomas. How-

1. Unna: The Histopathology of the Diseases of the Skin, 1896.



ever, it is possible that aside from causes acting locally a more general cause is to be found in a widespread abnormality of the vascular system. We availed ourselves of the method first described by Weiss<sup>2</sup> and later by Danzer and Hooker<sup>3</sup> to study the capillaries of the nailfolds of the fingers. The procedure is the following: The finger is placed on the stage of the microscope, keeping the arm in a comfortable position. We found it convenient to place the finger in a groove made in a flat cork, or in a tapering holder made of brass. The fingers have been scrubbed, dried and some cedar oil has been placed on the nailfold. A strong light is thrown on the nailfold. We used a 90 Watt nitrogen bulb and a lens of about seven inch focal length to concentrate the light on the finger. With a little practice

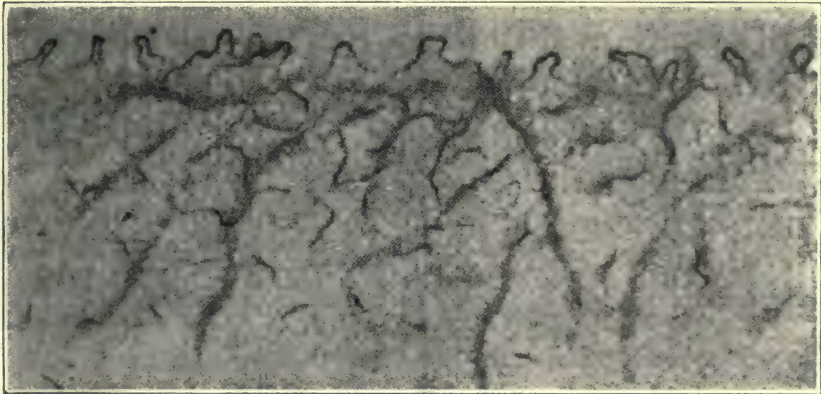


Fig. 3.—The degree of development of lymph vessels and blood vessels in early months of life.

it is very easy to find the last row of capillaries using the low power and preferably low eye-piece. The picture presenting itself is very pretty. As a rule, the capillaries appear in a row forming well defined slender loops in which the thinner arterial part is readily distinguished from the wider venous part. Sometimes the capillaries are smooth and evenly looped while others are tortuous and winding. Some form a figure 8, others are like corkscrews, etc.

Comparing the aspect presenting itself in our case with that of a number of children of various ages, and of adults, three striking

2. Weiss: Eine neue Methode zur Suffizienz pruefung des Kreislaufs. *Ztschr. f. Exper. Path. u. Therap.* **19**:437, 1918.

3. Danzer and Hooker: Determination of the Capillary Blood Pressure in Man with the Microcapillary Tonometer, *Am. J. Physiol.* **52**:136, 1920.

differences were noted: (1) the loops were very irregularly arranged; (2) the number of the loops was markedly greater than in other cases; and (3) the loops were less sharply defined and not well developed. They appeared to be stunted, that is, shorter and were more uniform, forming relatively broad, more or less curved, ends. The nailfolds of the toes showed the same picture as that of the fingers.

The aspect of the capillaries suggested that their development was arrested at an early stage. Holland and Meyer<sup>4</sup> studied the capillaries of a larger number of infants. They claim that the new-born arrives with an unfinished vascular system and uses its first months of life for the further development of its circulatory apparatus. Figure 3 is reproduced from their representation. It resembles somewhat the aspect of the capillary loops of our case. But in our case, the arrangement of the loops was much more irregular, the number of loops in a field was strikingly larger and their form showed even less variation. The observation of Mertz<sup>5</sup> corroborated those of Holland and Meyer, inasmuch as he saw during the first four to six weeks very short archlike loops protruding from the subcapillary vascular network. Some weeks later, the capillaries were developed further. Such pictures are not seen in every new-born. In older children and in adults they have not been described. According to Mertz, some individuals possess better developed capillaries than corresponds to the average of their age; in others, the development takes more time. In cases we have studied for comparison, including two premature infants, a number of infants during the first year of life and a large number of older children, we did not see such primitive capillaries. But Dr. K. Mayer, who continued these studies on a larger number of infants, has seen pictures coinciding with those described by Holland and Meyer, as well as by Mertz. In the two premature infants the capillaries were, comparatively speaking, remarkably well developed.

This much seems rather certain, that the capillaries of our case are abnormal for his age. We have to acknowledge the possibility that the abnormality of the vessels is a coincidence pure and simple and does not stand in any relationship to the local lesion.

But the presence of an abnormality of a part of the vascular system as in the case described, surely does suggest a relationship between

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4. Holland and Meyer: Beobachtungen an den Hautkapillaren bei Kindern mit exudativer Diathese, München. med. Wchnschr. **66**:1191, 1919.

5. Mertz: Beobachtungen an den Haut kapillaren von Saeuglingen, Monatschr. f. Kinderh. **18**:13, 1920.

this abnormality and the lesions. One can think of a direct causal relationship so that this abnormality of the vessels, in conjunction with some local factors, enters directly into the production of the lesion.

It may be said definitely that in cases of congenital angioma of the type described it is possible that a general abnormality of the vessels is one of the etiologic factors.

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## WATER RETENTION IN PNEUMONIA \*

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AND

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CHICAGO

It has been the custom at the Children's Memorial Hospital to keep daily weight records of all children under 2 years of age, if not suffering with a contagious disease. Dr. Amberg called our attention to the fact that children who keep up their weight or gaining weight during a febrile disease, may begin to lose weight with the disappearance of the fever. In some cases, this happened abruptly, in others gradually. A closer survey of the cases showed that the loss was more likely to occur in cases of lobar pneumonia. From the years 1914 to 1919 we were able to collect fifty-two cases of pneumonia suitable for our study. In all but one of the twenty-eight cases ending with a crisis, there was some loss of weight coincident with the crisis. This loss was frequently quite marked. The cases are recorded in Table 1. They represent uncomplicated cases. The table gives the age of the children, the duration of the disease before their entrance into the hospital, and the duration of the fever at the hospital. The loss of weight is recorded and the time during which it occurred. Figures 1 and 2 illustrate these conditions very well. In Figure 1 is recorded a loss of one pound; in Figure 2 is recorded a loss of 1 pound and 2 ounces within two days. The average loss of weight of the uncomplicated cases occurring within twenty-four hours, was  $9\frac{3}{5}$  ounces. The maximum loss in one day occurred in Case 41—11 $\frac{1}{4}$  pounds; the minimum, with the exception of Case 51 which was without loss, occurred in Case 2—6 ounces in two days.

We were unable to find any relationship between the degree and the rapidity of the loss to either the height of the temperature, as registered during the stay in the hospital, or to the duration of the fever. Neither was there a relationship between the range of the drop in temperature and the loss of weight. Unfortunately, in many instances, the data about the extent of the lung involvement are not sufficiently explicit; so we cannot say anything about a possible relationship between the amount of exudate and loss of weight. Figure 3 is of special interest. The child was brought in the day it was taken sick. On the third day

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\* From the Otho S. A. Sprague Memorial Institute Laboratory of the Children's Memorial Hospital.

\* Read before the Central States Pediatric Society, Oct. 25, 1919

of the disease an increase in weight began, lasting to the crisis on the seventh day. At this time, the weight dropped a little (about 4 ounces) and remained stationary. With an abrupt increase in weight, an edema of the eyelids and feet became manifest. This disappeared as rapidly as it had appeared. At the same time, the weight fell markedly.

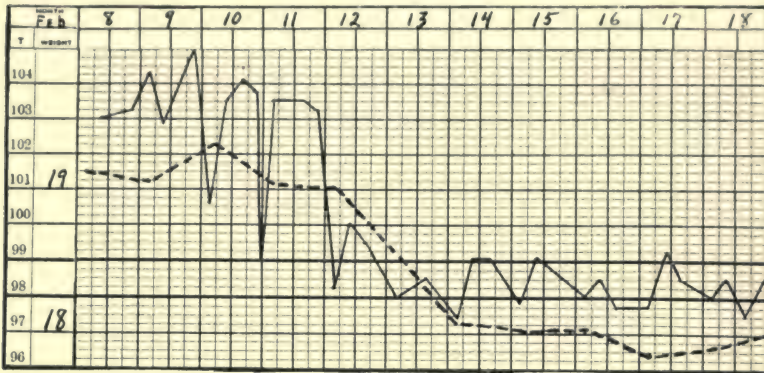


Figure 1

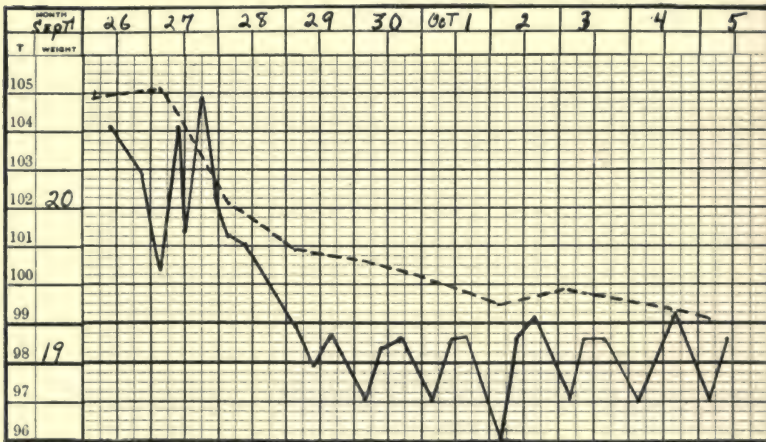


Figure 2

In Table 2 are recorded twenty-two cases of pneumonia which did not end in crisis. Twenty-one of the patients had some complication. In five, there was no loss of weight with the gradual defervescence, and when there was a loss of weight it never was abrupt, but extended over a considerable number of days.

Case 52 is the only uncomplicated case of lobar pneumonia with lysis. It presented a weight curve (Fig. 4) perhaps more in keeping

TABLE 1.—ANALYSIS OF TWENTY-NINE CASES OF PNEUMONIA

Case Number	Age, Months	Duration of Disease before Entrance, Days	Duration of Temperature in Hospital, Days	Definite Crisis	Complication	Loss of Weight	Time of Loss of Weight, Days
2	10	?	2	Yes	None	Ounces 6	2
4	7	1	1	Yes	None	12	3
9	7	7	8	Yes	None	Pounds 1½	3
11	4	4	4	Yes	None	1	3
15	18	4	12	Yes	None	2½	4
16	19	12	4	Yes	None	1	2
17	12	3	4	Yes	None	1½	3
19	14	1	3	Yes	None	1½	3
20	14	8	3	Yes	None	3½	4
21	16	2	4	Yes	None	¾	2
26	16	8	1	Yes	None	1	1
27	8	5	2	Yes	None	2½	3
28	12	14	3	Yes	None	2	3
29	13	3	3	Yes	None	1	3
32	6	3	3	Yes	None	¾	1
33	14	4	4	Yes	None	1¼	2
37	14	4	6	Yes	None	1½	2
38	8	2	6	Yes	None	1	1
40	3	3	3	Yes	None	¾	1
41	11	?	1	Yes	None	1¼	1
42	12	3	5	Yes	None	1½	2
43	18	3	5	Yes	None	3	2
45	9	6	5	Yes	None	1	1
46	14	3	5	Yes	None	1	1
47	5	3	6	Yes	None	1	1
48	11	4	3	Yes	None	1	1
50	10	3	3	Yes	None	1	1
51	11	4	3	Yes	None	1	2
52	11	8	8	None	None	Increase 1¼	12

TABLE 2

Case Number	Age, Months	Duration of Disease before Entrance	Duration of Temperature in Hospital	Definite Crisis	Complication	Loss of Weight, Pounds	Time of Loss of Weight
1	7	5 days	3 days	No	Otitis media	None	
3	10	4 days	7 days	No	Otitis media	None	
5	12	10 days	13 days	No	Enteritis	3½	Gradual
6	3	7 days	9 days	No	Otitis media	1¼	Gradual
7	11	6 days	3 days	No	None	1	Gradual
8	10	6 days	3 days	No	Otitis media	3	Gradual
10	16	?	5 days	No	Otitis media	None	
12	6	3 weeks	8 days	No	Otitis media	None	
13	12	4 days	3 days	No	Otitis media	None	
14	23	3 days	7 days	No	Meningitis	3	Gradual
18	9	3 days	6 days	No	Otitis media	1½	Gradual
22	19	2 days	8 days	No	Otitis media	¼	Gradual
23	8	3 days	6 days	No	Otitis media	1	Gradual
24	11	1 day	7 days	No	Otitis media	1	Gradual
25	13	8 days	9 days	No	Otitis media	¾	Gradual
30	13	6 days	3 days	No	Otitis media	1	Gradual
31	10	3 days	6 days	No	Otitis media	1½	Gradual
34	2	4 days	4 days	No	Otitis media	½	Gradual
36	14	4 days	4 days	No	Otitis media	1	Gradual
39	12	6 days	6 days	No	Otitis media	1½	Gradual
44	10	3 days	8 days	No	Empyema	2½	Gradual
49	18	7 days	4 days	No	Otitis media	2	Gradual



with what one would expect in a febrile disease associated with an increased metabolism. Here the drop in weight begins at the height of the fever before defervescence begins and proceeds rather uniformly.

Since the most frequent complication in our cases of pneumonia was otitis media, we were anxious to compare these cases with uncomplicated cases of otitis media. We were able to collect the data of eleven such cases, the ages of the patients varying from 4 to 13

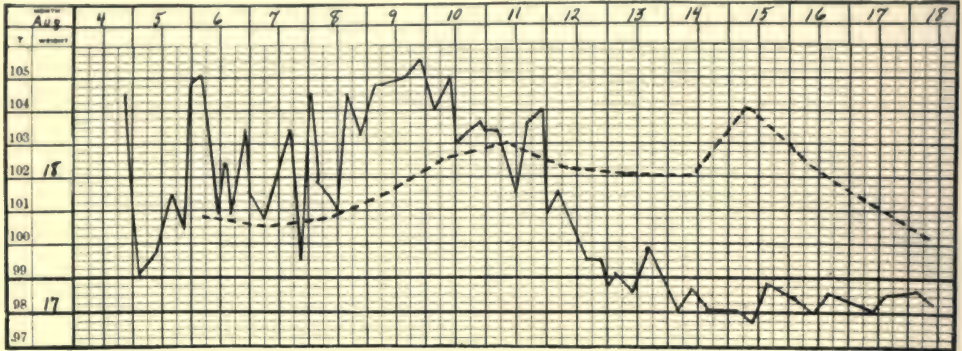


Figure 3

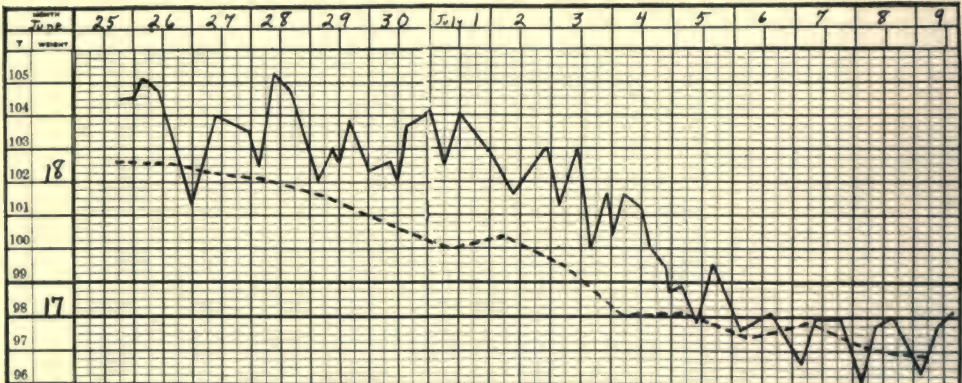


Figure 4

months. In nine of these cases (only one without paracentesis), the fall of the temperature was as abrupt or nearly as abrupt as in the case charted in Figure 5. In three of these cases there was a more or less marked drop of weight associated with the drop in temperature. In one instance, the weight dropped from 17 to 16 pounds and 2 ounces (a loss of 14 ounces); in another from 16 pounds and 9 ounces to 16 pounds and 1 ounce (a loss of 8 ounces) within twenty-four hours, and in a third case 8 ounces were lost from 13 pounds within two days. In



one case there was no loss of weight; in five cases the loss of weight, incidental to the drop of temperature, did not amount to more than 3 ounces. In two of these cases the temperature dropped very considerably, once from 104 to 98 F., and another time from 104 to 99 F. In two cases the temperature dropped more gradually, and in each case there was some loss of weight extending over several days, the loss, however, not being very marked.

The number of our observations is rather small; however, they indicate that cases of lobar pneumonia with crisis are more likely to be accompanied by a marked loss of weight coincident with or rather shortly preceding defervescence. In complicated cases, the weight curve dropped from the beginning of the observation with or without an acceleration of the drop during defervescence. Uncomplicated cases

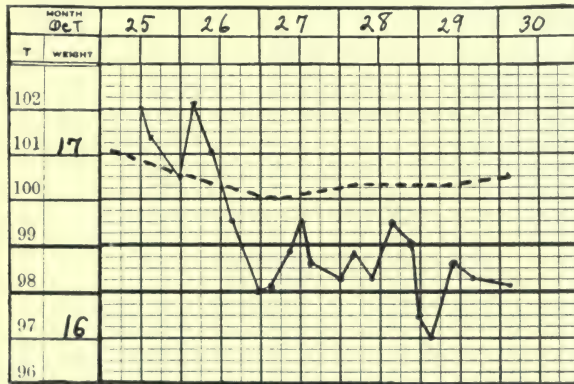


Figure 5

of otitis media may show a rather abrupt loss of weight with defervescence, but this seems to be more the exception than the rule.

Several factors may enter in this abrupt loss of weight in cases of pneumonia. It must be taken into consideration that a considerable amount of inflammatory exudate exists with this disease. Its rapid resolution has been shown to be associated with an increase in the amount of urine as well as in the amounts of the excreted chlorids and nitrogen, as shown by Cook.<sup>1</sup> The absorption and excretion of the inflammatory exudate or of its products may take part in this loss of weight. But this may not tell the whole story. Indeed, the occurrence of such abrupt losses of weight in some cases of otitis media shows that the resorption of the exudate cannot be the only factor concerned. Part, or even most, of this loss of weight may be due to an elimination

1. Cook, H. W.: Nitrogen Excretion in Pneumonia and Its Relation to Resolution, *Bull. Johns Hopkins Hosp.* **13**:307, 1902.

of previously retained water. Koeppe,<sup>2</sup> who observed an increase of weight of infants free from nutritional disorders during various febrile attacks, attributed this increase to retention of water.

That there is a marked change in the water economy of the body in pneumonia, as in other fevers, has been accepted by various authors. For instance, Fischer<sup>3</sup> states: "Edema is not an uncommon accompaniment of fever. In some fevers it constitutes a symptom so marked that it is looked for clinically; in others, the increased amount of water held by the patient is clearly indicated by his increase in weight and his failure to excrete an amount of water through kidneys, lungs, skin and bowel, the equivalent of that ingested. With remission or discontinuance of the fever there has been noticed by the most careful observers an increase in the output of water by all the water excreting organs above the amount ingested." Woodyatt<sup>4</sup> and his co-workers put it thus: "Everyone is familiar with the remarkable emptying out of water via the kidneys and skin which may follow the crisis in a case of pneumonia. Liters of water may thus be liberated in a few hours, giving visible proof of the water retention of the febrile stage."

In the case of pneumonia in children we may recall the fact that Maver and Schwartz<sup>5</sup> were able to demonstrate by means of the Schade elastometer, the presence of edema of the skin which could not be detected by simple palpation. Indeed, changes in the water distribution have been made responsible for the fever occurring under various conditions. The pediatrician is familiar with that conception, particularly through the contributions of Heim and John,<sup>6</sup> and of Peteri.<sup>7</sup> Woodyatt and his co-workers have presented this view in a recent paper, lending it new support by their experimental evidence. The sum and substance of this conception of the mechanism of fever is, that water, which is usually at the disposal of the organism for purposes of heat regulation, becomes not available for this purpose. This may occur by removing a certain amount of water from the organism, or by binding it within the organism in such a way that it cannot exercise its heat-dissipating function by evaporation from the body surfaces

2. Koeppe, H.: Studien zum Mineralstoffwechsel. (1) Wasserretention nach Ernährungsstörungen, *Jahr. f. Kinderheilk.* **73**:9, 1911.

3. Fischer, M.: Edema and Nephritis, 1915, p. 196.

4. Balcar, Sansum and Woodyatt: Fever and the Water Reserve of the Body, *Arch. Int. Med.* **24**:116 (July) 1919.

5. Maver and Schwartz: Studies in Edema in Pneumonia, *Arch. Int. Med.* **17**:459 (April) 1916.

6. Heim and John: Das alimentäre Fieber, *Ztschr. f. Kinderheilk.* **1**:398, 1911; Die Thermoregulation des gesunden und ernährungsgestörten Säuglings, *Jahrb. f. Kinderheilk.* **73**:266, 1911.

7. Peteri, I.: Beiträge zum pathologischen Wesen und zur Therapie des transitorischen Fiebers bei Neugeborenen, *Jahr. f. Kinderheilk.* **80**:612, 1914.

and lungs. In such instances it would be possible to have a water retention and still not enough for proper heat regulation. This may occur in a disease like pneumonia. With restoration of the water-binding power of the tissues to the normal, which may occur more or less abruptly, water would be liberated for heat regulatory purposes and elimination in urine. In this way, the loss of weight coincident or shortly preceding the abrupt fall of temperature, could be explained.

It must be kept in mind that our data are only such as have been obtained in the course of the hospital routine and do not justify us to do further than call attention to this very interesting subject.

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## DEXTROSE TOLERANCE IN ATROPHIC INFANTS\*

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CHICAGO

The tolerance of individuals to sugar has been determined previously by testing how much could be given by mouth or how much could be injected at one time subcutaneously or intravenously before sugar appeared in the urine. It was recognized that these methods were open to objections. In order to overcome these objections Woodyatt<sup>1</sup> constructed an apparatus permitting a continuous intravenous injection of solutions at any desired rate. The apparatus consists of a motor driven syringe filling and discharging automatically. In this way it was possible for Sansum and Wilder<sup>2</sup> to determine the tolerance of adults to dextrose much more accurately than had been possible before. They found that the appearance of the sugar in the urine depends on the amount of sugar injected in a unit of time, independent of the concentration of the sugar solution. The tolerance is expressed in grams of glucose which can be injected per kilogram of body weight in one hour at a uniform rate of flow, without leading to the excretion of sugar in the urine. For normal adults, dogs and rabbits this tolerance was from 0.8 to 0.9 gm. per kilogram per hour.

We determined the glucose tolerance of atrophic infants by this method with the idea in mind that such infants might derive some benefit from intravenous glucose injections.

The parts of the apparatus which come in contact with the fluid are sterilized by heat. A dextrose marked C. P., manufactured by the Corn Products Refining Company, was used. This is about 99 per cent. pure. The rubber tubing used is 8 mm. in diameter with a 3 mm. bore; a 50 c.c. graduated buret serves as a reservoir. A hand syringe is attached to the two-way stopcock inserted in the tubing between the pump and patient. A glass window connects the tubing with the 22 gage platinum or gold needle. In making the dilution we were guided by the fact that with our instrument the most convenient rate of injection was between 25 and 50 c.c. per hour. The glucose is dissolved in freshly distilled water and standardized on three successive days. The strength of the solution was made approximately 10, 15 and 20 per cent. The actual concentration was determined polari-

\*From the Otho S. A. Sprague Memorial Institute Laboratory, Children's Memorial Hospital, Chicago.

1. Woodyatt: *J. Biol. Chem.* **29**:355 (March) 1917.

2. Wilder and Sansum: *Arch. Int. Med.* **19**:341 (Feb.) 1917.

scopically. Buret readings were recorded every five minutes to detect fluctuations in rate. The hypertonic solutions did not cause perceptible changes in the erythrocytes.

The initial rate of injection was chosen just below the tolerance as established for normal adults. A rate of injection must be maintained for thirty minutes to insure a thorough saturation of the tissues before the urine is collected and tested for sugar. As Wilder and Sansum pointed out: "If the rate of injection only slightly exceeds the rate of utilization, glycosuria will occur within this time; if it does not, no glycosuria will occur even after several hours of continuous injection." If no urine is obtained after thirty minutes, the time of injection is lengthened until urine can be obtained. As a rule, the infants voided urine in from one-half to three-fourths of an hour. Catheterization after the beginning of an injection was occasionally resorted to if the infant did not urinate within a reasonable length of time. The urine was tested for sugar with Haines' solution, 1 c.c. of urine being taken for the test. If the urine did not contain sugar by injecting 0.7 gm. per kilogram per hour, the rate was increased to 0.8 gm. per kilogram per hour, and so on, increasing as a rule 0.1 gm. per kilogram per hour until sugar appeared in the urine. In working with the atrophic infants it was soon found to be unnecessary to begin below 1.0 gm. (The urine was always tested for sugar before the experiment was begun.) If sugar appeared in the urine on the first dosage, the experiment was discontinued for the day, as it was not possible to get the urine sugar free within a reasonable length of time. It must be stated, though, that the bladder was not washed out.

If sugar appeared at 1.0 gm. per kilogram per hour the tolerance was taken as 0.9 gm. per kilogram per hour; i. e., 0.1 gm. less than the amount given per kilogram per hour when sugar appeared. The injections were usually started two hours after a feeding, the infants being fed a simple milk dilution with the addition of dextri-maltose.

In the course of our work dextrose solutions were injected intravenously into four nonatrophic infants. These infants ranged in age from 5 to 15 months and were more nearly normal than any of the others. In these cases, the tolerance was found to be 0.8 to 0.9 gm. per kilogram of body weight per hour. These figures correspond very closely to the figures given by Wilder and Sansum for the normal adult. Table 1 gives a summary of these determinations.

The seven atrophic infants studied showed emaciation, tendency to subnormal temperature, lack of turgor and grayish color of the skin. Their weights were stationary or nearly so; the stools were good. In no case was the tolerance below 1.4 or 1.5 gm. per kilogram of

TABLE 1.—RESULTS OF INTRAVENOUS INJECTION OF DEXTROSE SOLUTIONS INTO NONATROPHIC INFANTS

No.	Date	Name	Age in Mos.	Weight in Kg.	Injection Time, Minutes	Gm. per Kg. per Hr.	Sugar in Urine
1	4/19	Tom D.	13½	7.65	30	0.8	0
					30	0.9	0
					30	1.1	—
2	4/12	Thad. G.	15	2.99	30	0.8	0
	4/16	.....	..	....	30	1.0	+
					30	0.9	—
3	5/16	Sophie	6	4.9	60	0.8	0
	5/23	.....	..	5.0	30	1.2	+
	5/28	.....	..	5.0	30	1.0	—
4	5/22	Norma	5	5.4	30	0.9	0
					30	1.0	+

TABLE 2.—RESULTS OF INTRAVENOUS INJECTION OF DEXTROSE SOLUTION INTO ATROPHIC INFANTS

No.	Date	Name	Age in Mos.	Weight in Kg.	Injection Time, Minutes	Gm. per Kg. per Hr.	Sugar in Urine
1	4/18/19	Francis	5	2.9	30	1.3	0
					30	1.4	0
					60	1.5	+
2	4/19	Mike L.	6	2.9	120	1.44	0
	4/24	.....	..	2.9	30	1.59	—
					90	1.2	0
					30	1.3	0
					15	1.4	0
					30	1.5	0
3	3/15/19	Stephen K.	6	3.18	30	1.1	0
					30	1.44	0
					30	1.66	+
4	3/31	Charles S.	7	3.46	30	1.7	0
					30	1.8	+
5	4/ 7	Leo L.	..	4.8	60	1.7	0
	4/ 8	.....	13	4.77	30	1.83	+
					30	1.43	0
					30	1.67	0
					30	1.63	0
					30	1.75	+
6	3/24	Viola G.	6	3.2	90	1.7	0
	3/28	.....	..	3.26	30	1.9	—
					60	1.8	—
					45	1.6	0
7	4/30	Marie L.	3½	2.5	30	1.0	0
					30	1.1	0
					60	1.3	0
	5/ 4	.....	..	2.87	60	1.6	0
					30	1.8	0
					30	1.9	+
	5/ 5	.....	..	2.87	60	1.6	0
					30	1.8	0
					30	1.9	+
	5/ 7	.....	..	2.87	30	1.8	0
					30	1.5	0
					60	1.7	0

body weight per hour (Table 2). Schlossmann,<sup>3</sup> Bahrt and Edelstein<sup>4</sup> and Murlin and Hoobler<sup>5</sup> found that the metabolism of the atrophic infant proceeded at a higher level than that of the normal infant. Observations of McClure and Sauer<sup>6</sup> have shown that atrophic infants have a higher surface temperature than normal infants and that there is an increased insensible perspiration. An increased sugar tolerance would seem to fit in very well with such observations.

In Case 7 (Table 2), Marie L., the injections were repeated a number of times for therapeutic reasons. It will be seen that the sugar tolerance in this individual under similar conditions is quite constant. In Cases 2, 5 and 7, and in two cases not included in this report, this same constancy was observed.

#### SUMMARY

1. The glucose tolerance of the approximately normal infant as determined by the Woodyatt method, is very likely identical with that of the normal adult which is 0.8 to 0.9 gm. per kilogram per hour.

2. The tolerance of atrophic infants for glucose is considerably greater: it varied in our cases from 1.4 or 1.5 gm. to 1.8 gm. per kilogram per hour.

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3. Schlossmann: *Ztschr. f. Kinderh.* **5**:227, 1912.

4. Bahrt and Edelstein: *Festschrift, Dr. O. Heubner*, Berlin, 1913.

5. Murlin and Hoobler: *Am. J. Dis. Child.* **9**:81 (Jan.) 1915.

6. McClure and Sauer: *Am. J. Dis. Child.* **10**:425 (Nov.) 1915. McClure and Sauer: *Arch. Int. Med.* **21**:428 (March) 1918.

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## THE TREATMENT OF PYELITIS IN INFANCY AND CHILDHOOD \*

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AND

HENRY F. HELMHOLZ, M.D.

CHICAGO

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This paper is based on a series of eleven cases treated by pelvic lavage with silver nitrate, and is intended as a preliminary report of one of our studies of pyelitis occurring in infants and children.

Nothing new is claimed for this method of treatment, that is, pelvic lavage, nor do we lay claim that silver nitrate is a specific, nor is it the only drug that may be or has been used in treating colon bacillus infections of the higher urinary tract. In this paper we wish to present the results obtained in this series of eleven cases that were treated by silver nitrate instillations into the renal pelvis.

The excellent results obtained in the treatment of *B. coli* infections in adults by means of silver nitrate injections of the renal pelvis seemed to justify an attempt to treat a series of cases in this way in order to determine whether or not this form of treatment could be applied to infants and children and, furthermore, to determine whether or not this method possesses advantages over the methods in general use, namely, internal treatment and vaccine treatment.

As a rule, pediatricians have not availed themselves of this form of treatment. The urologist who is prepared to carry out pelvic lavage does not, as a rule, see many of these cases. This situation calls for a closer cooperation between these two specialties than exists at present.

The acute hematogenous unilateral cases of renal infection, more or less fulminating in character, generally call for surgical intervention and will not be considered in this paper.

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There are several reasons why this form of treatment has not been employed in infants and children more frequently than it has. Perhaps the first of these is the lack of appreciation that this is possible, and the second, that general anesthesia is necessary.

Nitze,<sup>1</sup> Jacobi,<sup>2</sup> Casper,<sup>3</sup> Portner,<sup>4</sup> Beer,<sup>5</sup> Hyman,<sup>6</sup> Hinman<sup>7</sup> and Quinby<sup>8</sup> have called attention to the possibilities of cystoscopy in children. With the rapid advance in developing and perfecting small cystoscopes, this field has been very materially enlarged so that cystoscopy can be performed in infants as easily as in adults.

Nitze was the first to call attention to the use of cystoscopy and ureteral catheterization in children. He performed cystoscopy successfully in boys as young as 8 years of age. Naturally, because of anatomic conditions it is much easier to perform cystoscopy and catheterize ureters in girls at a much earlier age than it is in boys. Hyman reports a series of more than thirty children under 9 years of age in whom cystoscopy was performed. The youngest patient was a boy of 17 months who was subsequently operated on for kidney tumor. The youngest boy catheterized was under 3 years old, and the youngest girl was 22 months old. In our series the youngest patient was a girl 7 months old, in whom both ureters were catheterized simultaneously and pelvic lavage was carried out.

In boys, because of anatomic considerations, cystoscopy and ureteral catheterization cannot be carried out as early as it can be in girls, although one of us has repeatedly performed cystoscopy on boy babies of 14 months. In order to facilitate cystoscopy and ureteral catheterization in boys, it has been suggested that this may be done through an external urethrotomy incision. Thus far we have not availed ourselves of this procedure.

1. Nitze: *Lehrbuch der Kystoskopie*, 1907, p. 97.

2. Jacobi: *Lehrbuch der Kystoskopie*, Leipzig, 1911.

3. Casper: *Handbuch der Kystoskopie*, Leipzig, 1911.

4. Portner: *Deutsch. med. Wchnschr.*, 1908, No. 43.

5. Beer: *J. Surg.*, March, 1911.

6. Hyman, A.: *Surgical Diseases of the Urinary Tract in Children*, *Am. J. Dis. Child.* **15**: 116 (Feb.) 1918.

7. Hinman, Frank: *The Cystoscopic Study of Urologic Conditions in Children*, *Am. J. Dis. Child.* **17**: 305 (May) 1919.

8. Quinby, W. C.: *Pyelitis in Children*, *J. A. M. A.* **68**: 591 (Feb. 24) 1917.

As this paper deals with the treatment of pyelitis, no attempt will be made to include all of the cases in which cystoscopy has been employed from time to time for conditions other than those to be embraced in this paper.

The value of a routine roentgen-ray examination cannot be overemphasized. Experience in treating adults has demonstrated the value of this procedure before instituting pelvic lavage. In this way several cases of so-called pyelitis were proved to be cases of stone in the pelvis with infection, and hence the

TABLE 1.—FINDINGS IN CASE 1 (E. G., GIRL, AGED 5 YEARS)

Date	Leukoeyte Count			Cultures			Treatment
	Right	Left	Bladder	Right	Left	Bladder	
1/ 1/20	.....	...	200	.....	.....	B. coli	None
1/ 7/20	500	230	300	B. coli	B. coli	B. coli	
1/12/20	.....	.....	325	.....	.....	B. coli	
1/14/20	1,920	300	340	B. coli	Contam- inated	B. coli	
1/19/20	.....	...	130	.....	.....	B. coli	3 c.c. 0.5% silver nitrate, each pelvis
1/24/20	No spec- imen	30	...	.....	B. coli	B. coli	
2/ 2/20	.....	...	32	.....	.....	B. coli	2.5 c.c. 5% thor- ium sol. right pelvis; 3 c.c. in left
2/ 5/20	30	130	60	Sterile	Sterile	B. coli	
2/10/20	.....	...	3	.....	.....	Sterile	3.5 c.c. 0.5% sil- ver nitrate, lt. pelvis; 3 c.c. in right pelvis
2/16/20	.....	...	0	.....	.....	Sterile	

patients were spared a long and fruitless course of pelvic lavage. In one of the cases at the Children's Hospital in which the institution of pelvic lavage was considered, a preliminary roentgen-ray examination revealed the presence of a stone in the renal pelvis. Doubtless in some of the cases that are diagnosed as pyelitis and in which pelvic lavage fails to produce a cure, there are factors present which explain the failure, such as the presence of calculi, tuberculosis of the kidney, and stricture of the ureter.

In order not to overlook a possible renal tuberculosis, routine examination for tubercle bacilli were made, including guinea-pig inoculations from urine obtained from each kidney and bladder. In this series no evidence of renal tuberculosis was found.

The youngest patient treated was 7 months of age. In this case the two ureters were catheterized simultaneously, and both renal pelvises were treated with silver

nitrate. The oldest child was  $10\frac{1}{2}$  years old. Between these the following ages were recorded: one,  $11\frac{1}{2}$  years old; one, 2 years old; two,  $2\frac{1}{2}$  years old; one, 5 years old; one, 7 years old, and two,  $8\frac{1}{2}$  years old. All cases occurred in girls.

There were no untoward results or reactions following instrumentation and treatment. As a matter of fact, these little patients seemed to stand this quite as well as do adults.

It was our object in treating this series of cases to render the urine free of pus and sterile. In other words, no case was considered as cured in which these two requirements were not fulfilled. Symptomatic cures were not considered. It is a matter of general knowledge that there is a big difference between a symptomatic cure and a bacteriologic cure. Not infrequently these patients on admission to the hospital show a great deal of pus and countless numbers of bacteria in the urine. They have a very high temperature, a marked pallor is present, and they appear prostrated. Under routine hospital treatment, namely, rest in bed, bland diet, large amounts of water, the internal administration of alkalis, diuretics and urinary antiseptics, the fever disappears more or less rapidly, the appetite returns, and the patients make an apparently rapid recovery, so far as their general condition is concerned. This may at times occur as rapidly as if the patient had had a crisis, yet a careful examination of the urine discloses that the amount of pus and bacteria present in the urine is just the same after as it was before the clinical improvement took place. In other words, the clinical improvement is very marked, whereas the urinary findings remain unchanged or are only slightly improved.

It has been repeatedly stated by some authors that many of the cases of pyelitis of pregnancy and pyelitis of adolescence are but recurrences of the pyelitis of infancy and childhood. This may be possible, especially if we consider the fact that formerly these cases were treated without any consideration of the urinary findings or the bacteriologic results. As a matter of fact, some authors today are satisfied with a so-called clinical cure, paying little or no attention to the condition of the urine at the time the patient is discharged from treatment.



Thus, Rhonheimer<sup>9</sup> in his conclusions states that albumin, leukocytes, bacteria and epithelial cells may persist in the urine for a year after the acute illness, without necessarily considering these children as being sick, or that any special danger of recurrence persists.

With these views we can not agree. Hence we have not designated as cured any cases that showed either pus or bacteria in the urine.

In nine of the eleven cases, complete cures were obtained; that is, at the time the patients were discharged as cured, the urine was free of pus and the cultures were sterile.

TABLE 2.—FINDINGS IN CASE 2 (B. S., GIRL, AGED 8½ YEARS)

Date	Leukocyte Count			Cultures			Treatment
	Right	Left	Bladder	Right	Left	Bladder	
10/15/19	800	400	800	B. coli	B. coli	B. coli	3 c.c. 0.5% silver nitrate into each pelvis
10/27/19	...	...	870	.....	.....	B. coli	3 c.c. 0.5% silver nitrate into each pelvis
10/29/19	30	0	280	B. coli	B. coli	B. coli	
11/ 3/19	...	...	650	.....	.....	B. coli	2.5 c.c. 0.5% silver nitrate into each pelvis
11/12/19	...	...	650	.....	.....	B. coli	
11/19/19	240	120	450	B. coli	B. coli	B. coli	2.5 c.c. 0.5% silver nitrate into each pelvis
11/24/19	...	...	100	.....	.....	B. coli	
11/29/19	...	...	10	.....	.....	B. coli	2.5 c.c. 0.5% silver nitrate into each pelvis
12/ 3/19	0	0	10	Sterile	Sterile	Sterile	
12/ 8/19	...	...	0	.....	.....	Sterile	

The cultures were reported sterile if no growths were found at the end of forty-eight hours. In order, however, not to overlook the possibility of the presence of slow growing organisms, the plates were kept in the incubator for five days before a final report of a sterile specimen was given, so that it may be stated that in each case in which specimens were obtained, the cultures remained sterile at the end of the fifth day of incubation.

One patient left our observation after the first lavage. The cultures still showed the presence of infection; hence this case is not included under the cures, although the urine was free of pus.

Silver nitrate solution was used in each case. The strength of the solution used was 0.5 per cent. The

9. Rhonheimer: Cor.-Bl. f. Schweiz. Aerzte, Dec. 18, 1919.

amount injected varied from 1 c.c. in the infants to 5 c.c. in the older children. As stated above, there were no reactions.

The number of injections required to render the urine sterile varied. Three patients required but one injection of the silver nitrate. Five patients required two injections, and one patient required three injections.

In two cases the kidney urines were sterile before the bladder urine, in one case after two injections and in the other after the first injection. This patient had a *B. paratyphosus* infection. A subsequent examination one week after revealed that the kidneys were again infected.

In treating adults, this observation had been made several times. In some of the cases in which this fact was noted there were relapses or recurrences of the pyelitis. This fact would seem to be of sufficient importance to warrant emphasis because, if the kidneys show sterile specimens and the bladder still harbors infection, may not the bladder be a cause for subsequent recurrences or relapses? That this is possible, we believe. In a publication on cystography,<sup>10</sup> it was shown that fluid may regurgitate from the bladder up the ureter into the kidney pelvis. This phenomenon was noted in infants and children as well as in adults in a series of cases in which this procedure was carried out both in normal and in pathologic bladders.

Two cases in this series showed regurgitation of bladder fluid into the renal pelvis. This occurred during ureteral catheterization while waiting to collect specimens from each kidney. It was suddenly noted that the fluid ran out of the ureteral catheters very rapidly and that it was colorless. It was assumed that the water of distention in the bladder was flowing up the ureter and was running out of the catheters. In order to prove this assumption, argyrol solution was injected into the bladder, and in a few minutes the water flowing from the ureteral catheters was colored by the argyrol. Although catheters were in both ureters this phenomenon was present only on the left side. Several years ago Hagner<sup>11</sup> drew attention to this regurgitation during ureteral catheterization.

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10. Kretschmer, H. L.: Surg. Gynec. & Obst. 23: 709 (Dec.) 1916.  
 11. Hagner: Surg., Gynec. & Obst., October, 1912.

In order to differentiate between a true regurgitation due to insufficiency of the ureterovesical valve or sphincter and between a simple running up of fluid due to the presence of the catheter holding the ureterovesical valve open, the catheters were withdrawn from the ureters and the bladder fluid removed. The bladder was then filled with a 10 per cent. argyrol solution, and a roentgenogram was taken. In neither of these two cases after removal of the catheters did regurgitation up the ureter take place.

In ten of the eleven cases, the colon bacillus was found in pure culture. One patient had a paratyphoid bacillus infection.

In all of the cases the pyelitis was bilateral. There were no cases in which a colon cystitis was found; that is, in which only a bladder infection was present.

Routine leukocyte counts were made on each specimen of urine.<sup>12</sup> This method gives a more accurate estimation of the amount of pus present in the urine than does the use of indefinite terms now in use. Furthermore, when comparing the counts one can see at a glance just how much improvement is made from one treatment to the next. This can best be illustrated by the accompanying tables from two of the cases in this series.

#### SUMMARY

1. Pelvic lavage with solutions of silver nitrate is a procedure that can be carried out in infancy and childhood.
2. This mode of treatment has rendered the urine sterile and free of pus in nine cases in this series.
3. There have been no complications or unfavorable results of this treatment.
4. All of the cases treated this way had resisted all other forms of treatment.

12. Kretschmer, H. L.: The Value of Making Leukocyte Counts on the Urine in Infections of the Kidney, *J. A. M. A.* **69**: 1805 (N. Y.) 1917.





## THE INFLUENCE OF INTRAVENOUS INJECTIONS OF ACACIA-GLUCOSE SOLUTIONS ON URINE EXCRE- TION AND BLOOD VOLUME IN RABBITS

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The possibility of using acacia solutions intravenously in various clinical disorders suggested a series of animal experiments. We were particularly interested to learn how far acacia might be relied upon to keep up the blood volume in spite of a marked loss of water through the kidneys. Knowlton (1) found that both acacia and gelatin in 5 per cent solutions were effective in inhibiting saline diuresis. He believed the decreased secretion to be due to a change in concentration and therefore a change in the osmotic pressure of the blood colloids. The decrease in secretion is explained by the hypothesis put forth by Starling (2) in which the secretion of urine is regarded as a filtration process due to the difference between the capillary blood pressure and the osmotic pressure of the blood. Spiro (3) found in his study of the diuretic action of colloids, that their injection had but little effect on the output of urine in animals that had been starved from thirty-six to forty-eight hours prior to experimentation. In animals fed on a diet containing a high percentage of water, the injection of a 3 per cent gum solution caused an increase in urinary secretion. Moutard-Martin and Richet (4), in their experiments with sugar diuresis, observed that the sugar polyuria could be arrested by the injection of gum solution. They noted further that the decrease in urinary secretion following the injection of gum solution took place in spite of an increased blood pressure, whereas in the sugar polyuria the blood pressure was not increased, and might even be less than normal. Kruse (5)

states that urine secretion is diminished and at times nearly suppressed after acacia injections and he regards this as a factor in the maintenance of blood volume by acacia. He further states that the blood volume is well maintained, partly at the expense of urine secretion.

Our injections were given into the ear vein of rabbits. The experiment was so arranged that a given animal received on a given date an intravenous injection of glucose at the rate of 7 grams per kilogram of body weight per hour. After several days the same animal received a solution of glucose containing 6 per cent acacia, the glucose being given at the same rate as before. The urine was collected for the duration of the experiment—the bladder being emptied by expressing the urine at the beginning, at the end and one hour after the injection. Sometimes there was a variation in weights of the animals between the experiments, but it is doubtful whether this was enough to be of much influence on the results. We used glucose at this rate of injection for its powerful diuretic effect. The injections were given by a Woodyatt (6) motor-driven syringe.

In some preliminary experiments, comprising a series of eight rabbits, the injections were given for a period of one hour. This was found to be too short a time for the establishment of a marked diuresis in every case, so the time of injection was increased to one and one-half hours. The results of the latter experiments are given in table 1. The amounts of fluid injected and excreted, the difference between these amounts and the excretion per kilogram per hour is given in separate columns. In addition, hemoglobin determinations, made according to the Palmer (7) method are recorded. These readings were made at the beginning, at the end, and one hour after the close of the experiment. We were interested in the changes of hemoglobin in a given experiment, not in absolute values. The same standard was used for each experiment, but as the standards were kept for a week or more they may have changed, a possibility which has been pointed out by Cohen and Smith (8). Neither the injection of glucose nor of acacia-glucose leads to a withdrawal of red blood cells from the circulation as far as is known. The hemoglobin deter-

minations indicate, therefore, the changes in the blood volume. The last column of each table shows this percentage change. Our acacia solutions were not prepared with 0.9 per cent sodium chloride in place of water as Bayliss (9) demands, in order to make them isotonic with the red blood cells, but the glucose con-

TABLE 1

*Glucose*

NUMBER	WEIGHT	INJECTED IN	EXCRETED IN	EXCRETED IN	EXCESS EXCRETION IN	PER KILOGRAM AND HOUR (2½ HOURS)	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME IN 2½ HOURS
		1½ HOURS	1½ HOURS	2½ HOURS	2½ HOURS		Beginning of injection	End of injection	1 hour later	
	<i>kym.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.0	58	110	135	77	26	51	69	79	-3.5
2	1.7	56	150	150	94	35	83	63	100	-17
3	1.9	60	200	230	270	48	76	63	86	-11
4	2.02	56	200	200	144	40	86	100	111	-24
5	2.0	64	145	160	96	32	83	74	100	-17
6	2.07	64	150	150	86	31	83	86	96	-15
7	2.23	72	180	250	178	45	85	83	100	-15
8	2.46	74	180	220	146	36	71	78	83	-14
9	2.78	84	240	320	236	46	63	86	100	-33

*Six per cent acacia glucose*

1	1.87	56		90	34	20	75	50	72	+4
2	1.75	45		61	16	14	61	56	62	-2
3	1.8	68		210	142	46	75	71	65	+15
4	1.89	55		170	115	36	60	59	62	-3
4	1.95	68		118	50	24	71	48	71	0
5	1.75	45		100	55	23	83	82	83	0
6	2.19	53		180	127	32	71	71	72	-2
7	2.165	52		190	138	35	72	64	70	+2.5
8	2.14	60		150	90	28	64	61	63	+2
9	2.67	61		?	—	—	77	78	78	-1

centrations, between 30 and 40 per cent, were such as to make our solutions markedly hypertonic.

A rule, the urine excretion from glucose alone, in these nine animals exceeded that following acacia-glucose. The preliminary series of eight animals referred to above, also showed, on the whole, some diminution in the urine output with acacia-glucose when compared with the glucose alone. The changes in the



blood volume as shown by the hemoglobin readings at the end of the glucose experiments were variable; in some the blood volume was increased, in others there was a decrease. The percentage increase or decrease of the blood volume was calculated, using as a basis a blood volume equal to 5.5 per cent<sup>1</sup> of the body weight. At the end of the injection the blood volume varied between a concentration of 26 per cent and a dilution of 32 per cent. But, without a single exception, the readings one hour after the injection showed a decided decrease in the blood volume. This concentration varied between 11 and 35 per cent. It is therefore clear that when glucose is given alone the withdrawal of fluids leads to a diminution of the blood volume.

With the injection of 6 per cent acacia and glucose into these animals, the urine output was more or less diminished in all but one compared with the urine output of the same animals with glucose injection. The hemoglobin determinations showed a transitory dilution of the blood in about one-half the cases—this with one exception had disappeared almost entirely one hour later, the final readings being almost identical with the initial readings. The increase in blood volume amounted in two cases close to 50 per cent; in the others it varied between zero and 12 per cent. One hour later it was 15 per cent in one case, in the others it varied but little from the initial. This is in marked contrast to the concentration observed in the glucose experiments. This happened in spite of a quite marked diuresis as may be seen in the table.

The nine glucose animals of table 1 serve also as controls for the experiments recorded in tables 2 and 3.

Table 2 gives the results of 12 per cent acacia-glucose injections in six rabbits. The amount of urine excreted is variable—in animal no. 2 there was none at all. In nos. 4 and 5 the amount was great. There is no very marked difference in the output of urine when compared with that of the animals receiving 6 per cent acacia-glucose. In these experiments the urinary output was only recorded during the period of injection. The blood vol-

<sup>1</sup> Meek and Gasser (10) give the blood volume of the rabbit at 5.44 per cent of the body weight.



ume is much more uniformly increased than in the other series and more persistently so. The blood volume had increased at the end of the injection from 8 to 62 per cent; one hour later it had slightly decreased in one instance, in another it had practically returned to the starting point, in the rest it had increased from 18 to 50 per cent. In no. 5 with a high urine excretion, there was a slight concentration one hour after close of the experiment and but little more on the following day (twenty hours later). In animals nos. 4 and 6 there was a marked dilution which persisted, though to a less degree on the following day. In no. 2 no urine was voided nor could any be expressed. The result of this experiment is puzzling. We included it in our

TABLE 2  
*Twelve per cent acacia-glucose*

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.4	72	90	18	25	66		50	+32
2	2.17	62	0			56	50	57	-1
3	1.9	60	90	30	32	64	50	54	+18
4	2.17	81	220	139	53	68	42	50	+36
5	2.62	78	270	192	69	69	64	71	-3.5
6	1.71	52	140	88	55	75	51	50	+50

series because we could find no justification for not doing so. It is to be noted that only one animal excreted urine within the next twenty-four hours.

Table 3 gives the results obtained when 3 per cent acacia-glucose is injected. The urinary excretion is not inhibited, in fact the output per kilogram per hour at the end of injection exceeds even that of the control animals in which glucose alone was used. The blood volume, in spite of marked diuresis, remains fairly constant. This is also in marked contrast to the concentration seen when glucose alone is used. At the end of the injection the blood volume was definitely increased twice (10 and 24 per cent). In the other four cases there was little change. After one hour

it was increased 6 per cent in one case, in the remainder it varied but slightly from the initial volume.

It must be stated that no changes indicative of kidney lesions were found either before or after the experiments, but with two exceptions. No. 2 of table 1 showed hyalin casts after 6 per cent acacia-glucose and in no. 7 of table 4 albumen was found after 3 per cent acacia saline. Neither did the animals show any marked clinical symptoms during the injection nor afterwards. At the end of the glucose experiment in no. 3 of table 1 there were some respiratory changes. All animals were quiet after injections, food being refused until the following day. All lived in apparently good health for several weeks. We tried to deter-

TABLE 3  
*Three per cent acacia-glucose*

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.24	67	210	143	62	82	66	81	+1
2	1.86	54	135	81	51	58	52	56	+3
3	1.88	57	160	103	57	71	71	76	-4
4	1.39	42	165	123	79	62	63	63	-3
5	2.27	69	185	116	54	68	70	71	-4
6	2.03	60	180	120	59	72	71	68	+6

mine whether or not acacia was excreted in the urine by the furfural phloroglucin method. The large and varying amounts of pentosans present in normal rabbits' urine made this impossible.

The simultaneous injection of 6 or 12 per cent acacia and glucose may exercise an inhibitory effect on the glucose diuresis. This effect seems to be variable and not constant. Acacia given in a 3 per cent solution cannot be said to have exercised any inhibitory effect on the glucose diuresis. Indeed it is questionable whether it did not enhance somewhat the diuretic effect of the glucose. An idea of the degree of diuresis obtained with glucose in our experiments may be gained from the fact that the amount of urine excreted over and above the amount of solution injected

approached or even exceeded the total calculated blood volume of the animal. In some of the glucose acacia experiments the same holds true. But while the glucose acacia experiments are followed by a marked concentration of the blood volume, the acacia-glucose injections are not. In spite of a very pronounced diuresis the blood volume is maintained. In all but two cases (table 1, no. 9 and table 2, no. 2) the amount of urine exceeded the amount of fluid injected. In most instances this excess was considerable, and no doubt much greater than the amount of urine which would have been produced spontaneously. Fluid must enter the vessels and is held therein in spite of the diuresis.

TABLE 4  
*Three per cent acacia in normal saline*

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.23	61	36	-25	6.4	56	50	42	+30
2	2.9	80	40	-40	5.2	57	54	50	+14
3	1.8	50	64	+14	14.0	100	83	100	0
4	1.7	48	70	+22	16.0	93	83	86	+7
5	1.56	44	5	-39	1.2	116	85	88	+32
6	3.2	90	94	+4	12.0	72	63	64	+12
7	2.6	74	30	-44	4.6	78	69	72	+8
8	2.46	69	65	-4	10.0	83	71	78	+6

These results support the supposition of Gasser and Erlanger (11) that glucose brings fluid into the circulation and is held there by the acacia. The possibility that the 3 per cent acacia favored diuresis has been noted. Indeed, Spiro, it will be remembered, claimed a diuretic effect of 3 per cent gum solution in animals having received a diet rich in water. In his experiments the gum was dissolved in saline solution, but the injections were given at a rate much exceeding ours.

Our results with 3 per cent acacia in physiological salt solution obtained in eight animals are recorded in table 4. We injected about half of the calculated blood volume within one and one-half hours. In another, smaller series of animals represented by table



5, physiological salt solution alone was given at the same rate. All the animals were on a diet rich in water. In only three of the eight rabbits receiving the acacia did the output of urine exceed the amount of fluid injected, and in only one of the three animals receiving saline alone. Even with this small number of controls it can be said that 3 per cent acacia has no very definite diuretic effect under the conditions of our experiments.

It is impossible to bring the changes of the blood volume in our experiments in direct relation to the renal activity. A great deal of stress has been laid on the importance of osmosis for the maintenance or increase of blood volume by acacia. So Gasser, Erlanger and Meek (12) state that the injection of acacia greatly reduced the transudation of plasma which occurs in traumatic

TABLE 5  
*Normal saline*

NUMBER	WEIGHT	INJECTED IN 1½ HOURS	EXCRETED IN 2½ HOURS	EXCESS EXCRE- TION IN 2½ HOURS	PER KILO- GRAM AND HOUR	HEMOGLOBIN PER CENT			PER CENT CHANGE OF BLOOD VOLUME
						Begin- ning of injection	End of injection	1 hour later	
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>				
1	2.82	80	30	-50	4.2	96	79	78	+22
2	2.37	67	35	-32	6.0	83	75	77	+6
3	2.4	68	70	+2	11.6	65	63	55	+18

shock. This is one of the main factors in the decrease of the blood volume. The mechanism of its action is seen in the antagonism to filtration by the resulting increase in the osmotic pressure of the plasma colloids. Nevertheless these authors remark that after the injection of 4 cc. per kilogram 25 per cent acacia solution in the dog the maximal actual expansion of the blood never reaches anything like the theoretical calculated from the osmotic pressure of such a solution. This point, they say, is of considerable interest as it minimizes the importance of what would seem to be one of the most obvious explanations of the protective action of acacia, namely, its increase in volume by the attraction of water. Some of our results make us also hesitate to stress too much the importance of the osmotic explanation of the maintenance of blood pressure by acacia. We calculated the



osmotic pressure of the serum of our animals one hour after the end of the injection from the changes in blood volume, adding to it the osmotic pressure derived from the amount of acacia injected. We utilized for this purpose the figures of Gasser, Erlanger and Meek (11) who give the osmotic pressure of dog serum (measured with the aid of a colloidin membrane) as 16.4 mm. Hg., and that of a 7 per cent acacia solution as 22 mm. Hg. Comparing the calculated osmotic pressure in mm. Hg with the increase or decrease in blood volume, no relationship was discernible. True, this calculation of the osmotic pressure can only be done very roughly. It was assumed that neither protein nor acacia leave the circulation. We are aware that the latter is really not the case as Meek and Gasser (10) pointed out. We realize that the conditions of these experiments do not entitle us to any definite opinion. All we can say is that the results make one hesitate to accept the osmotic pressure as the all-important factor in the maintenance of the blood volume by acacia. This doubt is strengthened by the experiments with 3 per cent acacia and saline and with saline alone. In the latter the dilution of the blood cannot be dependent in any way on the osmotic pressure.

#### CONCLUSIONS

Acacia is capable of maintaining the blood volume in spite of a very marked glucose diuresis. Injecting acacia-glucose mixture wherein the glucose is present in concentrations of 30 to 40 per cent in quantities of about one-half of the rabbit's blood volume in one and one-half hours a decrease of the blood volume is practically prevented by the presence of 3 per cent acacia in the injection fluid. Glucose injections without acacia produce uniformly a diminution of the blood volume.

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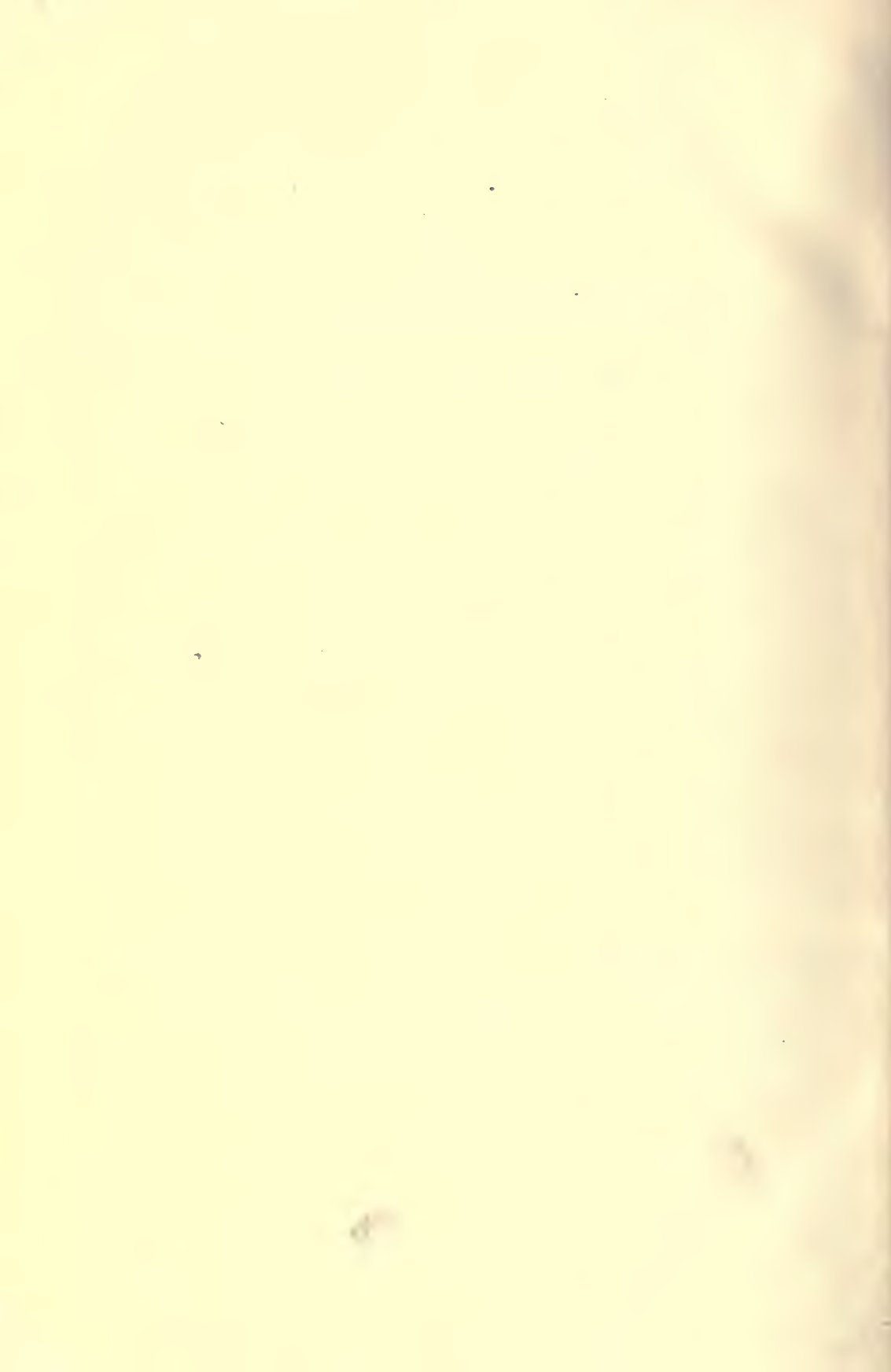
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PATHOLOGIC ANATOMY OF TRAUMATIC  
FRACTURES OF CRANIAL BONES

AND CONCOMITANT BRAIN INJURIES



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# PATHOLOGIC ANATOMY OF TRAUMATIC FRACTURES OF CRANIAL BONES

AND CONCOMITANT BRAIN INJURIES \*

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This attempt to consider the injuries of the brain and cranial bones when the latter are broken by external violence is based on conditions encountered in 504 postmortem examinations made by one of us during the years 1911 to 1918. It does not include all the postmortem examinations during that period of the bodies of persons with such traumatic fractures, for in about sixty instances the measurements and other steps necessary in the interests of precision were not so detailed as in the 504 here reviewed. The patients were cared for in the Cook County Hospital or the Hospital of the House of Correction, and some post-mortem examinations were of bodies of persons who were found dead or who died en route to a hospital.

## SIX FUNDAMENTAL CONSIDERATIONS

Notwithstanding certain still mooted questions regarding the mechanism whereby the injuries of both the cranial bones and the brain are produced, and especially the influence a whirling motion of the falling head and body may have on the characteristics of the injuries, there are six facts that are generally accepted and need brief mention as a background for what follows:

1. There are six regions where the greater thickness of the cranial bones forms arches thicker below and gradually thinning out in the vault: one in front from the root of the nose and glabella; one behind, including theinion and the external and internal crests of the occipital bone, as well as the torcular eminence; one on each side from the external angular processes of the frontal bone and prolonged obliquely back and into the body of the sphenoid in the bones of the skull base, and finally one on each side formed by the petrous

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\*From the Coroner's Medical Service at the Cook County Hospital.

\*Aided by a grant from the Otho S. A. Sprague Memorial Institute.

bones and continued externally in the protuberance of the mastoid (Figs. 1, 2 and 3).

2. With violence applied to the cranium, these arches hinder horizontal bending; whereas the bone between the arches can more easily bend vertically. As a result, the bone between the arches flattens the more in a horizontal plane and breaks across, the linear fractures radiating up into the vault and down into the bones of the base of the cranium between the arches.

3. With the head in motion, the brain lags behind the more rapidly moving cranium, and as a consequence is closer to the cranial bones *opposite* where violence is applied; and with the axis of the skull abruptly shortened at right angles to where violence is applied, the brain is the more bruised opposite that place.

4. The resistance (weight) of the trunk and extremities transmitted to the skull via the condyles of the occipital bone tends to bend in the bottom of the skull, especially the arches, and is one factor determining the course and distribution of the fractures.

5. When the cranial bones are broken with the head in a fixed position, contrecoup bruising of the brain is reduced to the minimum and the bruises are direct, at the place of fracturing.

6. The general direction taken by fractures which course between the arches is also well known, and is shown in Figures 4, 5, 7, 10, 19, 23 and 29.<sup>1</sup>

#### NATURE AND LOCATION OF FRACTURES

About 85 per cent. of the 504 cases here considered were simple linear fractures or linear fractures with branches; in the remainder, the bones were extensively comminuted and with some of the fragments depressed. With the bones of the cranial base involved slightly more than those of the vault, both were involved in varying degrees in all but about 8 per cent. of the 504. When grouped according to the fossae chiefly involved, with one group for the vault and one for fractures so extensive that several fossae and part of the vault as well were broken, the incidence is as shown in the accompanying table. In this table are included a few fractures partly healed or so healed that their courses could still be followed. Many of the fractures of the back fossae ran forward to end in the ethmoid bone or one of the foraminae of the

1. We have made no attempt to cite from the literature, but wish to refer especially to the work of Tilmann (Arch. f. klin. Chir. **66**:750, 1902; **59**:236, 1899) and that of M. Auvray (Maladies du crâne et de l'encéphale, Paris, 1909).

middle fossae. Violence for these fractures as indicated by the scalp injuries was usually to the back of the head. Of the sixty-one fractures of the anterior fossae, seventeen were simple linear, from 2 to 5 cm. long and in the roof of one orbit. Of the extensive fractures, usually with comminution, the violence was to the back of the head for twenty-seven, to the side for nineteen and in front for four. The average of the total linear length of these fractures was 70 cm., the greatest 138.9 cm. (Fig. 19). With the vault fractures, some traumatic diastasis of sutures was not rare: in twelve of the forty-nine, part of the sagittal or its entire length was affected; in three, the coronal suture, and in five, one or both lambdoid sutures. As regards the meningitis, the petrous parts of the temporal bones were the portal of entry for the infection

LOCATION OF FRACTURES

	Pos- terior Fos- sae	Mid- dle Fos- sae	An- terior Fos- sae	Vault	Ex- ten- sive	Totals
Total number.....	178	166	61	49	50	504
Some depression.....	6	11	6	4	7	34
Mode of injury learned.....	130	124	51	33	42	380
Short falls.....	36	43	9	2	2	92
Longer falls.....	48	37	13	16	13	127
Street car.....	17	12	16	4	13	62
Automobile and autotruck..	11	17	7	2	11	48
Assault.....	18	15	6	9	3	51
Meningitis.....	15	7	13	3	1	39
Healed fractures.....	7	6	4	1	0	18
Decompression operation.....	15	27	3	8	6	59

in twenty-three, the ethmoid bone in thirteen; in two fractures the opened sagittal suture with the scalp laceration, and in one the petrous bone of one side was broken and a decompression operation had been made. With thirteen of the eighteen fractures with some degree of healing, there were contrecoup bruises, and in four, direct bruises alone. With one of these healed fractures there was an abscess of the left cerebral hemisphere in front, the fracture of the right posterior fossa and no outside bruise of the brain; in other words, the abscess opposite the fracture.

#### CONCOMITANT BRAIN INJURY

The injuries of the brain concomitant with fractures of the cranial bones are preponderantly of the outside of the brain, owing partly to the inbending of

the cranial bones, but chiefly to the bumping of the brain against the cranial bones. Most fractures of the cranium result from causes which first set the head into rapid motion and then suddenly stop the skull against a firm object. The brain, floating as it does



Fig. 1.—Location of the reinforcing arches of the cranium.



Fig. 2.—The thickest portions of the cranial bones are in the shaded areas.

in the cerebrospinal fluid, lags in the movement of the head, so much so that when the head stops moving the brain receives its greatest injury by bumping against the cranial bones directly opposite where the cranium is broken. When the bones of the temporal fossae or of the nuchal planes of the occipital bones are frac-



tured, because of their thinness, there is frequently a bruise directly under the fracture resulting from inbending of the bone and compression of the brain. The back half of the cerebrum is larger and heavier than the front half, and because of this the front poles of the cerebrum are more frequently and more extensively bruised than the back poles. If the lateral ventricles are enlarged (internal hydrocephalus), the sides of the brain and front poles are more easily torn. As a result of these factors the frontal and temporal lobes are the most frequent site of injury; the injuries of the back of the head have a large percentage of contrecoup bruises; injuries of the sides of the head, the next largest percentage of contrecoup bruises; and injuries of the front of the head are more frequently associated with large direct bruises than with large contrecoup bruises.

#### DEGREES OF BRAIN INJURY

That the degree of brain injuries may be indicated in some way, they are roughly divided into seven groups, according to severity:

1. Severe lacerations of the brain from 4 to 6 cm. in diameter and extending into the brain 4 or 5 cm. Here there is a defect in the surface filled with clotted blood and torn brain tissue. There were fifty-four injuries of this type, and in fifteen the tear had extended into one of the lateral ventricles (Figs. 14 and 22). In thirty-nine of the fifty-four, bleeding continued intracerebrally for a few centimeters, so that in places on some of the surfaces made by coronal sections of the brain beyond the margins of the bruises of the outside there were hemorrhages inside of the brain not connected with the surface (Figs. 12, 13 and 14). Thirty-six of the latter occurred in the frontal or temporal lobes, three in the occipital lobes, and usually with severe injuries (street car, automobile, high falls, etc.). There were subdural hemorrhages with all of the fifty-four injuries, weighing from 10 to 200 gm. All except eight were contrecoup injuries.

2. Bruises of the brain, usually wedge-shaped, in which the brain is infiltrated with blood from 2 to 4 cm. deep in a place 4 or 5 cm. in the largest outside dimensions (Figs. 25, 30 and 32). The leptomeninges covering the bruise are usually torn, the brain is lacerated from 1 to 2 cm. deep, the adjacent tissue is thickly infiltrated with blood, and the margins are edematous and dotted with petechial hemorrhages. Subdural hemorrhage is always present, but in amounts varying from a few grams up to 100 gm. Bruises of this type were the most frequent of all of the larger bruises. There were 248 in this series: 182 contrecoup and sixty-six direct.



Fig. 3.—As the arches come together at the body of the sphenoid bone they are curled up at their ends; because of this, fractures radiating down into the base of the cranium course toward the body of the sphenoid bone, as shown in many of the illustrations.

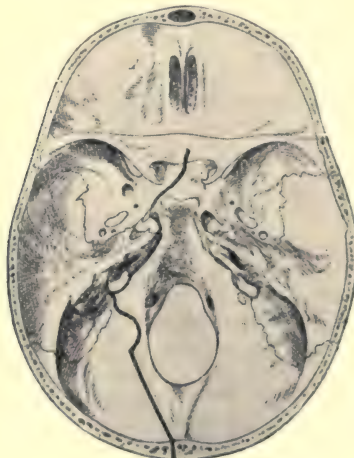


Fig. 4.—The fractures shown in this illustration and in Figures 5, 6 and 7 may be taken as "composites," that is to say, they represent conditions with extension from the vault down into the different fossae. (Figures 1, 3, 4, 5, 6 and 7 are taken from LeDentu, A., and Delbet, Pierre: *Nouveau traité de chirurgie*, XIII. Auvray, M.: *Maladies du crâne et de l'encéphale*, Paris, 1909.)

3. Bruises, frequently with the leptomeninges intact, from 1 to 1.5 cm. deep, with little laceration of the brain tissue. There is generally hemorrhage into the brain, wedge-shaped on cross-section (Figs. 11, 16, 24, 25, 27 and 28). These are found with cranial fractures in which the trauma is usually of such a degree as might result from a short fall on a sidewalk, floor, etc. They are also frequently associated with the severe injuries, mentioned above, that is, with a blow on the back of the head on the right side the large bruise will be of the left frontal or temporal lobe, while one of these more superficial bruises will be found of the right frontal and temporal lobes or of the right side of the cerebellum (Figs.

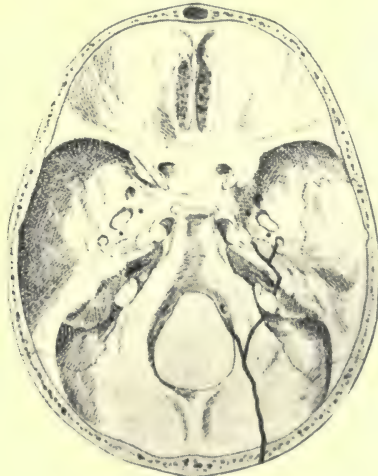


Fig. 5.—Composite fracture.

11, 24 and 25). This type of injury was present with about 35 per cent. of all the fractures. In only fifteen when these were the largest bruises present were there subdural hemorrhages with clots weighing more than 10 gm., and in only two did they weigh as much as 90 gm.

4. Superficial contusions represented by closely set petechial hemorrhages of the cortex in places from 1 to 1.5 cm. in diameter (Figs. 21, 24, 27 and 32). The meninges are intact and usually there is a little bleeding into the leptomeninges about the contusion. One or more of these contusions is usually present when the brain is severely injured, oftentimes five or six. Like those in Class 3, they are in those parts of the brain some distance away from the direct line of force (Figs. 16, 21 and 24). Some of them were the only gross evidence of direct injury of the brain.

5. Hemorrhages into the pons and medulla, chiefly the pons, centrally located, often multiple and the individual hemorrhages from 1 to 3 mm. in diameter (Fig. 18). These are usually contrecoup in location, that is to say, the fractures do not course through the bones adjacent to the brain-stem. The explanation of these hemorrhages is variously stated: contrecoup bruising, stretching of the brain away from the brain-stem because the former is more movable, and interference with the blood supply of the pons and medulla (infarction).<sup>2</sup> There were eighteen brains with hemorrhages in the brain-stem with fractures of the posterior fossae: in thirteen multiple, and in five a single hemorrhage from 2 to 4 mm. in diameter. In thirteen of the 166 fractures of the middle fossae, there were hemorrhages in the pons; ten of these



Fig. 6.—Composite fracture.

patients lived less than one day, one three days, one seven days and one ten days. There were three with fractures of the frontal fossae, and all were associated with extensive injury of the brain. There were brain-stem hemorrhages in eighteen of the fifty extensively comminuted fractures; twelve with the violence applied behind, five of the side and one of the front of the skull. In the forty-nine fractures of the vault, there were hemorrhages in the brain-stem in five, and none of these patients lived longer than one day in the hospital.<sup>3</sup>

2. Greenacre, Phyllis: Multiple Spontaneous Intracerebral Hemorrhages, a Contribution to the Pathology of Apoplexy. *Bull. Johns Hopkins Hosp.* 28: 312, 1917.

3. With some of these hemorrhages, sugar is found in the urine, as it is also with large spontaneous (apoplexy) hemorrhages in the pons.



6. Small intracerebral hemorrhages, usually in the cerebral basal ganglions, from 5 to 10 mm. in diameter. There were only six of these in the 504 fractures of this series (Fig. 18).

7. Small intracerebral hemorrhages about 1 cm. in diameter, usually single, occurring when an extradural blood clot compresses one of the hemispheres. They are always in the compressed hemisphere. They were present with only four of the 104 large extradural hemorrhages (Fig. 30).

#### INCIDENCE AND NATURE OF BRAIN INJURIES

*Brain Injuries with Fractures of the Posterior Fossae.*—In 149 (83.70 per cent.) of the 178 fractures, the largest bruise of the brain was contrecoup, in sixteen (8.98 per cent.) direct; and in three both the contrecoup and direct bruises were of equal extent.

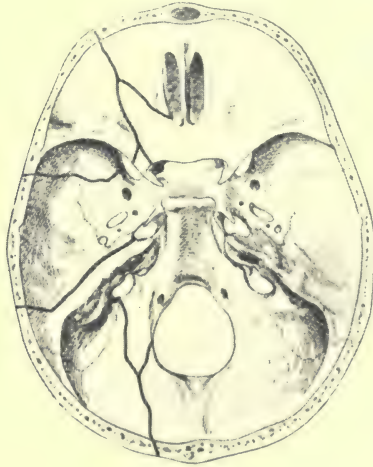


Fig. 7.—Composite fracture.

In the remaining ten the brain injury was slight. When the fracture was near the midline, the bruising was usually fairly symmetrical of each frontal lobe at the tips, lower outer margins and of the tips of the temporal lobes (Figs. 20, 21 and 22). With the fractures coursing more outward from the midline and forward in the posterior fossae, the contrecoup bruises were chiefly of the opposite side of the brain (Figs. 10, 11, 12 and 13). As a rule with the violence applied close to the back of the ear, the frontal and temporal lobes on that side were little if any bruised.

In 159 of this 178 (89.32 per cent.), one or both frontal lobes bore bruises, tears or both; in 120 (67.41 per cent.), the temporal lobes had so suffered; in sixteen (8.98 per cent.), the occipital lobes; and in fifty-five (30.89 per cent.), the cerebellum. As already stated, the injuries of the frontal and temporal lobes were chiefly of their frontmost convolutions, undersurfaces and lower convolutions; but with the point of injury high on the back of the head, the undersurfaces of these parts of the cerebrum were chiefly bruised; with the injury at the level of the external occipital protuberance, the contrecoup bruises were chiefly of the frontal poles of the cerebrum; if at some distance from the midline, the outer margins of the opposite frontal or temporal lobes were chiefly bruised; in short, there has not been observed any noteworthy deviation from the directly opposite location of the

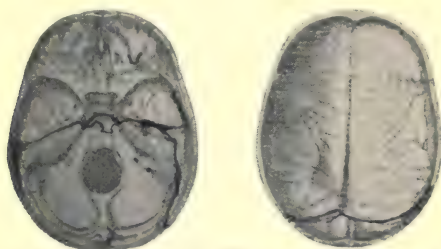


Fig. 8 (Case 23).—An extensive, traumatic, comminuted fracture of the cranial bones with an independent fracture of the roof of the right orbit. The fracture is 53 cm. long. The scalp was bruised opposite the right mastoid region. This man, aged 52, was struck by a street car six hours before death.

contrecoup bruising of the brain which is such a conspicuous and important feature of the lesions accompanying the fractures.

*Brain Injuries with Fractures of the Middle Fossae.*

—In 103 (62.04 per cent.) of the 166 fractures, the largest bruises of the brain were contrecoup, in forty-three (25.90 per cent.) direct. In the remaining twenty the brain injury grossly visible was only slight leptomeningeal hemorrhage of about equal extent on the two sides of the brain, except in a few brains which were without gross injury. In seventy (42.16 per cent.) of this 166, one or both frontal lobes bore bruises, tears or both; in 146 (87.94 per cent.), one

or both temporal lobes; in thirty-eight (22.89 per cent.), the parietal lobes; in fifteen (9.03 per cent.), the occipital lobes, and in ten (6.02 per cent.), the cerebellum. The bruises of the frontal lobes were mostly of the outer margin and undersurface. In only a few instances were the bruises of the frontal lobes deep, and then the external injury was of the back half of the parietal region of the opposite side. The bruises of the temporal lobes were preeminently of the outer margin and undersurface, depending on whether the violence was applied low or high on the vault, respectively (Figs. 23, 24, 26 and 27). One or both of the temporal lobes were bruised whenever the brain was injured.

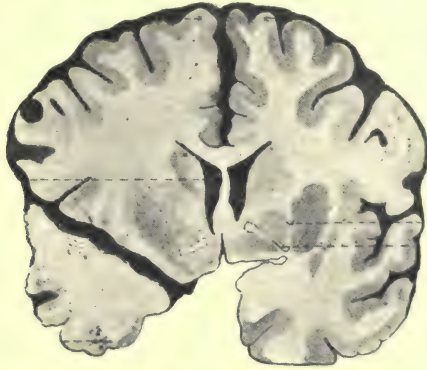


Fig. 9 (Case 23).—Extensive traumatic leptomeningeal hemorrhage due to extension from contrecoup bruises of the frontal and left temporal lobes.

*Bruises of the Brain with Fractures of the Frontal Fossae.*—In thirty-five (57.37 per cent.) of the sixty-one, the greatest bruising of the brain was direct, in thirteen (21.31 per cent.) contrecoup. In forty-four (72.13 per cent.), the frontal lobes were bruised or torn; in seventeen (27.70 per cent.), one or both temporal lobes; in three (4.91 per cent.), one or both parietal lobes; in nineteen (31.14 per cent.), one or both occipital lobes; and in eleven (18.03 per cent.), the cerebellum. In thirteen brains there was no gross evidence of injury, and in thirty-seven of the sixty-one fractures the brain injury was slight (or no injury was present), death resulting from other causes (in nine, from meningitis; in eight, from delirium tremens;

in six, from broken bones; in five, from epilepsy; in two each, from bronchopneumonia and internal injuries; and in one each, from uremia, tetanus, drowning, syphilis and so-called pachymeningitis hemorrhagica interna).

*Brain Injuries with Extensively Comminuted Fractures of the Cranium.*—The greatest bruising of the brain in thirty-one (62 per cent.) of the fifty was contrecoup; in fourteen (28 per cent.), direct; and in five, both the contrecoup and direct bruises were equally severe. In only two of the fifty brains were the injuries slight, consisting of direct leptomeningeal hemorrhage. The point of external violence was used in deciding whether the bruising was contrecoup or direct, because some of the fractures encircled the skull, and especially through the middle fossae. In thirty-nine of the fifty (78 per cent.), one or both

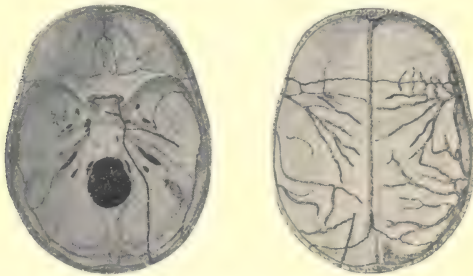


Fig. 10 (Case 24).—Typical linear fracture of the right posterior fossa resulting from the patient's falling backward and striking the back of the head on a cement floor. The patient was a white man, aged 45.

frontal lobes were bruised or torn; in thirty-seven (74 per cent.), one or both temporal lobes; in seven (14 per cent.), one or both parietal lobes; in eight (16 per cent.), one or both occipital lobes; and in sixteen (32 per cent.), the cerebellum. Of the twenty-seven fractures in which the violence was applied behind, the contrecoup bruising was greatest in fourteen; in four, direct; in eight, chiefly contrecoup, but the direct bruises were also large, and in one there was little injury of the brain. Of the nineteen with the violence applied to the side of the skull, in nine the contrecoup bruising was greatest, in five, direct, and in five chiefly contrecoup; but the direct bruises were also large. With the external violence applied in front, the direct



bruises were greatest in three of the four, and in one the brain injury was slight.

*Brain Injuries with Fractures of the Vault.*—In thirty-nine (79.59 per cent.), the greatest bruising was contrecoup; in nine (18.36 per cent.), direct; and in one, the brain injury was slight. In eight of the thirty-nine brains, the contrecoup bruising was only slightly greater than the direct. The frontal lobes were bruised or torn in thirty-nine (79.59 per cent.) of the forty-nine fractures; the temporal lobes in thirty-three (67.34 per cent.); the parietal lobes in sixteen (32.65 per cent.); the occipital lobes in nine (18.36 per cent.), and the cerebellum in six (12.24 per cent.). The contrecoup bruises were mostly of the undersurfaces of the frontal and temporal lobes, usually with many contusions of the type included in Class 4 (Figs. 31 and 32).



Fig. 11 (Case 24).—The location of the contrecoup tears of the left frontal and temporal lobes (Class 1), the superficial contusion of the right frontal lobe (Class 3) and the extent of traumatic leptomeningeal hemorrhage. The front half of the left cerebral hemisphere was covered by a subdural clot, and at the tears the clot was from 8 to 10 mm. thick. Altogether the subdural clot weighed about 25 gm.

#### SUBDURAL TRAUMATIC HEMORRHAGES

Subdural traumatic hemorrhages result most frequently from lacerated cerebral veins where the brain is bruised. Also of importance are the bleeding, torn, cortical vessels. Infrequent sources of subdural hemorrhage are torn dural venous sinuses and the larger cerebral arteries of the base of the brain. In order to obtain facts for a better understanding of the location and extent of subdural hemorrhages, as much of the blood as could readily be removed from the torn brain

without further injury was weighed, its distribution measured and the injury described, all with some accuracy.

*Subdural Hemorrhages with Fractures of the Posterior Fossae.*—Examinations were made in 136 of the 178 fractures of this group. In 115 of the 136 there was subdural hemorrhage, 104 contrecoup and eleven direct. In seventy-one of the 115 the blood weighed between 20 and 210 gm., but the most frequent weight of these large clots was about 40 gm. In the remaining forty-four there were usually only a few grams (from 1 to 10 gm.), and in a few from 10 to 20 gm. Of the large subdural hemorrhages, fifty-four were local, that is, confined to the front third or front half of the cranial cavity; seventeen

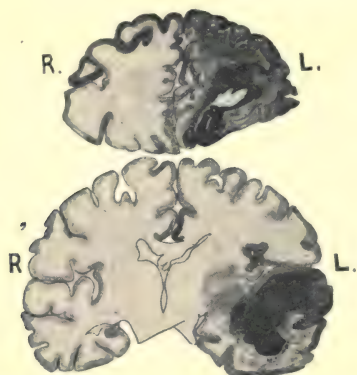


Fig. 12 (Case 24).—Coronal sections (anterior surface) through the frontal and temporal lobes showing the depth of the contrecoup bruising (Class 1).

were more widely spread over one side of the cerebrum. Of these large hemorrhages, sixty-six were altogether on one side. Two of the large subdural hemorrhages were contributed to by tears of large cerebral veins without severe injury to the brain.

*Subdural Hemorrhages with Fractures of the Middle Fossae.*—Examinations were made in 134 of the 166 fractures of this group. In ninety-eight there was subdural hemorrhage. Of these, in fifty-one the blood weighed between 20 and 310 gm., in the other forty-seven much less. In sixty-three the subdural hemorrhage was contrecoup, in thirty-five, direct. Of the

large subdural hemorrhages, thirty-six of the fifty-one were contrecoup, and fifteen, direct. Also, thirty-one of the large subdural hemorrhages were local, and twenty widely spread. The small subdural hemorrhages were only a few millimeters thick and extended from 1 to 10 mm. beyond the margins of the bruise from which the bleeding occurred. With increasing size, the subdural hemorrhages spread mostly up over the parietal lobe, the front margin advancing less than the upper and back margins.

*Subdural Hemorrhages with Fractures of the Frontal Fossae.*—In twenty-one of the forty-eight accurately described cranial injuries in this group there

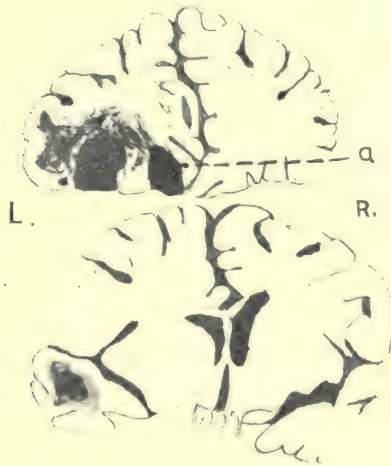


Fig. 13 (Case 25).—Coronal sections (posterior surface) through the frontal and temporal lobes showing contrecoup bruises of the left frontal lobe, Class 1, and intracerebral bleeding back into the left temporal lobe.

was subdural hemorrhage, and of these twenty-one, thirteen were direct and eight contrecoup. There were only six large subdural hemorrhages, five direct and one contrecoup, the latter beneath the tentorium cerebelli.

*Subdural Hemorrhages with Extensively Commi-nuted Fractures.*—In forty-three of the fifty fractures in this group, accurate records of the subdural hemorrhage or its entire absence were made. There were subdural hemorrhages in thirty six, twenty large and

sixteen small. Eighteen of the large subdural hemorrhages were contrecoup, and only two direct. Of the sixteen small hemorrhages, twelve were contrecoup and four direct. In several instances there were both direct and contrecoup subdural hemorrhages.

*Subdural Hemorrhages with Fractures of the Vault.*  
—There were subdural hemorrhages in thirty-eight of the forty accurately described brains; twenty-eight of these were large and ten small. All but three of the large subdural hemorrhages were contrecoup, and fourteen of the large hemorrhages were local.

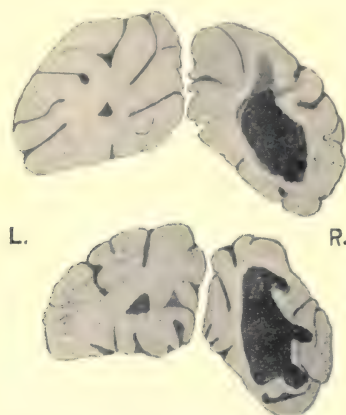


Fig. 14 (Case 25).—Coronal sections (posterior surface) through the occipital lobes. The bruise of the outside could not be seen from the undersurface with the cerebellum attached (Class 1) and both intracerebral bleeding beyond the margins of the bruise and rupture into a ventricle.

#### TRAUMATIC EXTRADURAL HEMORRHAGES

Bleeding, which splits the dura away from the cranial bones as a result of fracture, may result from laceration of the trunk or branches of one of the middle meningeal arteries, the dural venous sinuses, the cerebral veins at the point of entrance into the superior longitudinal sinus, tears of the dura allowing free subdural blood to enter the extradural space, from the broken cranial bones, or from pericranial hemorrhages with comminuted depressed fractures.

There were extradural hemorrhages in 199 (39.48 per cent.) of the 504 fractures in this series. Of these 199, 104 (52.26 per cent.) were large enough to produce appreciable compression of the brain (from 20 to



246 gm.) and ninety-five were small, usually only a few grams. Of the large extradural hemorrhages, seventy-three (70.19 per cent.) were with fractures of the middle fossae; fifteen (14.42 per cent.) with fractures of the posterior fossae; ten (9.61 per cent.) with fractures of the vault; three (2.88 per cent.) each with fractures of the frontal fossae and fractures listed as extensively comminuted. About 50 per cent. of the small extradural hemorrhages resulted from

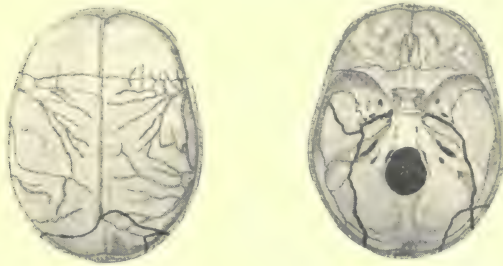


Fig. 15 (Case 26).—Extensive, traumatic, comminuted fracture of the cranial bones, 55.9 cm. long. The cause of the fracture was undetermined. The scalp bruises were opposite the lambda. The patient was a white man, about 35 years of age.

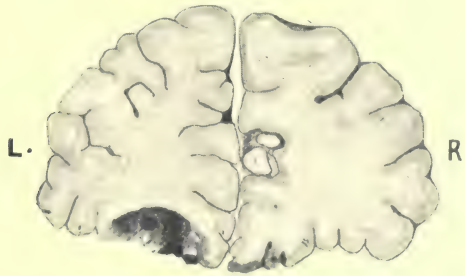


Fig. 16 (Case 26).—Coronal section (posterior surface) of the frontal lobes. Contrecoup bruises of Class 3 of the left frontal lobe and Class 4 of the right frontal lobe are shown here.

bleeding of the broken bones, and 20 per cent. from torn dural sinuses. The remainder were probably due partially to bleeding from broken bones and from pericranial hemorrhage.

Of the large extradural hemorrhages, forty-nine covered the temporal and parietal lobes on one side, thirty-four the temporal, parietal and occipital lobes on one side, fourteen one occipital lobe, six the

frontal and parietal lobes on one side, and one the temporal and parietal lobes of both sides. Of these large hemorrhages, forty-nine were attributed to bleeding from the anterior branch of one of the middle meningeal arteries, forty-four from one of the posterior branches, three to laceration of the superior longitudinal sinus (the blood in two weighed between 25 and 30 gm., in the third about 60 gm. on each side, that on the left removed by the surgeon) and eight, in which there were fractures of the posterior fossae, from one of the transverse sinuses, the blood in none of the latter weighing more than 50 gm. Thirty-six of the large extradural hemorrhages were with fractures of the middle fossae passing through the anterior parts of the fossae in the squamous portions of the temporal bones, and thirty-seven with

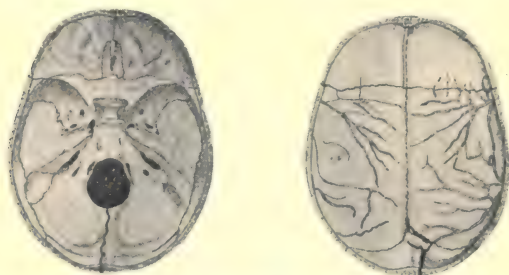


Fig. 17 (Case 27).—Healing, traumatic fracture of the skull of undetermined cause, in a negro, aged 40. This illustrates the course of many fractures of the posterior fossae.

fractures that passed through the middle and posterior portions of the fossae. Comminution of the bones in the middle fossae occurred in less than half of the seventy-three large extradural hemorrhages; most of the fractures were of the linear type. Fifty-four of the large extradural hemorrhages were on the left side, forty-nine were on the right side, and one was on both sides.

The usual shape of the blood clot of the large compressing type is oval, the margins thinnest and the center thickest. The long dimension is obliquely directed up and back. The lower border is seldom lower than the middle temporal convolution, and the upper border is usually 1 or 2 cm. from the superior longitudinal sinus (only in six instances did the clot

reach to the sinus). The thickest portion of the clot is usually at the middle of the outer surface of one of the parietal lobes so that the greatest compression of the brain is in a line drawn horizontally through the middle of the parietal eminences. The compression of the brain is saucer-shaped (Fig. 30). The blood clots early, so that with death one or one and one-half days after the injury the clot adheres to the dura, when the calvarium is removed, rather than to the bones. Later the clot becomes so adherent that it is necessary to use the sharp edge of a knife to detach it. The average dimensions of a clot weighing about 100 gm. (this is an average weight) are 10 by 8 cm. and from 3 to 3.5 cm. thick.

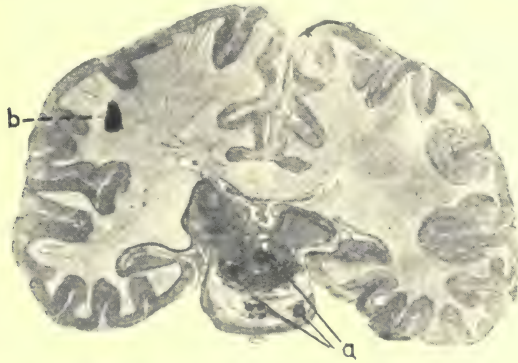


Fig. 18 (Case 27).—Typical traumatic hemorrhage (a) in the pons (Class 5), and (b) traumatic hemorrhage of the intracerebral type (Class 6), usually in the cerebral ganglions.

In nine of the large extradural hemorrhages caused by fractures of the middle fossae, there was no bruising of the brain grossly visible; in thirty-three there were only superficial bruises like those included in Class 3 of brain injuries; in twenty-five there was sufficient bruising to allow from 5 to 20 gm. of blood to enter the subdural space, and in six there were large subdural clots weighing from 40 to 120 gm. In none of the fifteen large extradural hemorrhages resulting from fractures of the posterior fossae was the brain without bruises, and in thirteen there were large bruises, contrecoup, and in the other two like those of Class 3. There were bruises of the brain in the remainder of the large extradural hemorrhages, fre-

quently like those of Class 2. The proportion of contrecoup and direct bruises was approximately the same in fractures with large extradural hemorrhages as with the fractures without such hemorrhages.

There were decompression operations in twenty-three of the large extradural hemorrhages, in some with free blood still present at the time of the post-mortem examination in and beneath the decompression defect, and weighing from 60 to 120 gm. There were several decompression operations not included in these twenty-three in which the conditions postmortem precluded conclusions as to whether a large extradural clot was present before the operation; and for these no mention of a large extradural hemorrhage was made

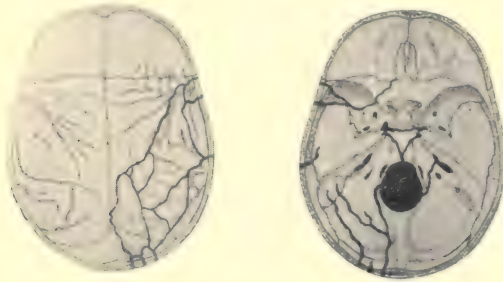


Fig. 19 (Case 28).—Extensive comminuted, traumatic fracture of the cranial bones, 138.9 cm. long, in a white man, aged 52, who, while in the hospital suffering from chronic alcoholism, jumped from a second story balcony to the main floor and struck the cement floor head first. There were superficial contrecoup contusions of the brain (Classes 3 and 4) and moderate leptomeningeal hemorrhage. Death occurred in five minutes.

in the description of the operation by the surgeon. Sixteen of the twenty-three operations were with fractures of the middle fossae, three with fractures of the posterior fossae, three with fractures of the vault, and one with an extensively comminuted fracture.<sup>4</sup>

#### TRAUMATIC LEPTOMENINGEAL HEMORRHAGE

Bleeding into the subarachnoid space or between the brain and pia (subpial) occurred in about 95 per cent. of all the fractures. Subarachnoid hemorrhage results

4. Although the period of time covered in the two articles is the same, a little discrepancy exists between the figures regarding extradural hemorrhages by us and the figures of Dr. Moody (*THE JOURNAL*, Feb. 21, 1920, 74: 511). This is due to the inclusion here of cases from the Hospital of the House of Correction.



most frequently from bleeding of bruises of the brain, but also from rupture of leptomeningeal vessels. Subpial hemorrhage results from tears of the pial arteries and cortical vessels. Subpial hemorrhage is seldom as extensive as subarachnoid hemorrhage, and is usually only enough to discolor the surface of the brain pink.

The extent and amount of subarachnoid hemorrhage is dependent on the size of the tear of the arachnoid membrane at the bruise (with large defects, bleeding occurs more readily into the subdural space<sup>5</sup> than with smaller defects in which the margins of the bruise extend beyond the edges of the arachnoid tear), the bruise of the brain and the laceration of leptomeningeal vessels. Therefore, the amount of subarachnoid hemorrhage is not always proportional to the extent of the bruising of the brain. It is always thickest



Fig. 20 (Case 29).—Typical linear fracture of the midline of the posterior fossae, 21.2 cm. long. The patient, a white woman, aged 38, died about four days after falling down a flight of stairs.

about the margins of the bruises and gradually thins out centrifugally, sometimes covering an entire hemisphere. When extensive, from 1 to 3 mm. thick, the convolutions of the brain are hidden. When less extensive, the sulci stand out as a network because the blood tends to collect in them, and between them the convolutions form a pink meshwork. When slight, the convolutions are discolored pink ("traumatic lividity"). Although severe brain injuries and extensive leptomeningeal hemorrhage are frequently coincident, still there were a few extensively comminuted frac-

<sup>5</sup> Strictly speaking, the term "subdural space" is a misstatement because the inside of the dura is lined with the smooth, glistening parietal layer of the arachnoid, and for "subdural space," which is commonly used, there should be substituted "arachnoid space."

tures associated with superficial bruises of the brain but with extensive leptomeningeal hemorrhage, and with some of these death occurred abruptly—from within a few minutes to several hours (Fig. 9). The location of leptomeningeal hemorrhages corresponds in general to that of bruises in the various types of fractures.

#### TRAUMATIC EDEMA OF THE BRAIN

The most frequent change in brains of patients dying from fracture of the skull was traumatic edema of the brain, and it was the only change sufficient to explain death in a few cases. As in other forms of edema of the brain, the convolutions are flattened, the cerebral veins relatively empty and flattened, the peripheral ends of the sulci closed up more or less tightly, the fluid in the leptomeninges greatly lessened; and when the edema is marked, the visceral layer of the arach-



Fig. 21 (Case 29).—The location and extent of the contrecoup tears (Class 1) of the frontal lobes (a) with rupture of the lateral ventricles, contusions (Class 4) of the temporal lobes (b) and traumatic subdural hemorrhage (c). A thin layer of clotted blood in the subdural space covered the entire brain, and at each tear there were about 5 gm. Altogether the subdural clot weighed 23 gm.

noid is almost dry when the dura is first removed, and by reflected light this surface of the arachnoid is finely granular because the little moisture present is heaped up by separating the two serous surfaces into almost microscopic droplets.

#### REPORT OF CASES

The failure on the part of police officers and physicians to recognize the presence of fractures of the cranial bones and severe brain injury, or to recognize these injuries only after several hours, requires some

comment. Some of the cases reported concern the transfer of patients with fracture of the cranial bones from one hospital to another, in a few even after the nature of the injury was known.

CASE 1.—A man, aged 45, who had fallen on a sidewalk, was taken to a police station and remained there from 11 p. m. until 12:15 p. m. the next day, when he was taken to the Cook County Hospital, where cerebral hemorrhage, left sided hemiplegia and basal skull fracture were diagnosed. He lived forty-five hours in the hospital.

Postmortem examination revealed a fracture of the right side of the vault, 17 cm. long, below this an extradural clot weighing 118 gm., covering the right motor region, and superficial direct contusions of the right cerebral hemisphere. The only external sign of injury was a bruise of the scalp about the right ear.

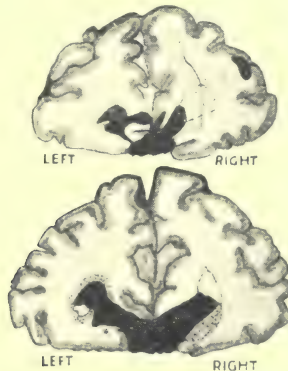


Fig. 22 (Case 29).—Coronal sections (posterior surface) through the frontal lobes, showing the depth of the tears and the petechial hemorrhages and edema of the surrounding brain tissue.

CASE 2.—An adult negro, after a street fight, while intoxicated, fell to the sidewalk and was taken to a police station, where he remained about twelve hours, lying on a cement floor. The following morning he was taken home, and then worked for three days as a porter in a saloon. On the fourth day he became "dopy" and was taken to the Cook County Hospital, where cerebral hemorrhage and lacerated scalp were diagnosed. He lived eleven days in the hospital, and five days passed between the time of injury and the entrance to the hospital.

Postmortem examination disclosed a fracture of the left half of the posterior fossa, 8 cm. long, contrecoup contusions of the frontal and temporal lobes, that of the right frontal 5

cm. deep, a huge subdural blood clot weighing 120 gm. of the front half of the right subdural space (brown and clotted firmly), and a healing laceration of the scalp near theinion.

CASE 3.—A man, aged 37, walked into a police station at 9 p. m., supposedly drunk. The next morning at 10:30 a. m. he was taken to the Cook County Hospital, where he lived two and one-half days, cerebral hemorrhage and lobar pneumonia being diagnosed.

At the postmortem examination a fracture of the right half of the posterior fossa, 32.8 cm. long, extensive destruction of the tip of the right frontal lobe, 3 cm. deep, and a thin clot of blood in the subdural space covering the whole right hemisphere, but chiefly in front, were found. There was no sign of external injury of the scalp.

CASE 4.—A man, aged 50, fell down a flight of stairs about 1 a. m. and arrived at one police station at 2 a. m. the same day, where he remained for twenty minutes and was then taken to another police station for medical care, remaining



Fig. 23 (Case 30).—Traumatic, slightly depressed, fracture of the skull, 19.5 cm. long. On the right side in the parieto-occipital region there was an extradural clot, 9 by 7 by 1 cm. in its largest dimensions, and weighing 40 gm. The patient, a white boy, aged 14 years, was struck by an automobile seven and one-half days before death.

there until 8:40 a. m. the same morning, when he was taken to the Hospital of the House of Correction. Skull fracture was diagnosed and two decompression operations performed, one of each parietal region. He lived for three and one-half days in the hospital.

Postmortem examination revealed a linear fracture of the left posterior fossa, 9 cm. long, contrecoup bruises of the frontal and temporal lobes, that of the right frontal lobe being deepest, reaching to the lateral ventricle, a thin subdural clot covering the front half of the right cerebral hemisphere, and an extradural clot weighing 40 gm. in the operation defect of the left parietal region.

CASE 5.—A man, aged 22, after an unknown injury, and supposedly suffering from alcoholism, was taken to a hospital where he remained one day before skull fracture was



suspected, after which he was taken to the Hospital of the House of Correction, where he lived two days. Skull fracture was diagnosed and a decompression operation of the left parietal region was performed.

Postmortem examination revealed a fracture of the left posterior fossa, 34.8 cm. long, contrecoup bruises of the right frontal and temporal lobes, and a direct bruise of the left half of the cerebellum, a large subdural clot on the right side in the front half, an extradural clot filling the decompression bone defect and a bruise of the scalp behind.

CASE 6.—A man, aged 35, arrested and taken to a police station, remained twelve hours, suffering from alcoholism and epileptic fits, according to the police officers. He was taken to the Hospital of the House of Correction, where he lived fifteen hours. Skull fracture was diagnosed.

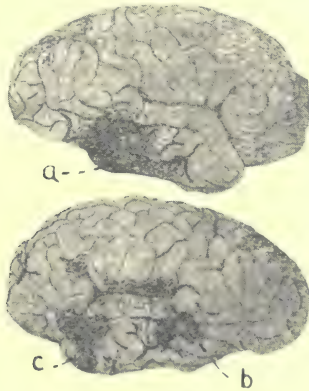


Fig. 24 (Case 30).—A direct bruise at *a* (Class 2) under the depressed fracture, a contrecoup bruise at *b* (Class 3), and superficial contusions at *c* (Class 4). About the bruises there was leptomeningeal hemorrhage. There were several grams of blood subdural about the tear of the right temporal lobe, but none on the left side.

Postmortem examination revealed an extensively comminuted fracture of the back and base of the skull, with diastasis of both lambdoid sutures, altogether 60 cm. long; also there were deep contrecoup bruises of the frontal poles, subdural clots covering both frontal poles, 30 gm. of blood in the decompression defect of the left parietal region, and bruises of the scalp behind.

CASE 7.—A man, aged 40, was arrested and taken to a police station on account of alcoholism; after remaining there twelve hours he was taken to the Hospital of the House of Correction, where he lived twenty-eight hours, and where a decompression operation of the left parietal region was performed.

Postmortem examination revealed a fracture of the right parietal bone, slightly comminuted and 17 cm. long, a large contrecoup bruise of the right frontal lobe, blood subdural in front on the right side weighing 110 gm., and bruises of the scalp over the fracture.

In these cases there was failure to detect the presence of a skull fracture, or the fracture was discovered only after hours or days had elapsed:<sup>6</sup>

CASE 8.—A man, aged 28, was knocked to the ground by a falling sack of malt weighing 200 pounds. He was taken home, where he remained three days. Several physicians examined him but none of them suspected the presence of a fracture of the skull, and one sent him to the hospital for the insane, where he lived seven hours and fifty minutes. No diagnosis was made in the hospital.

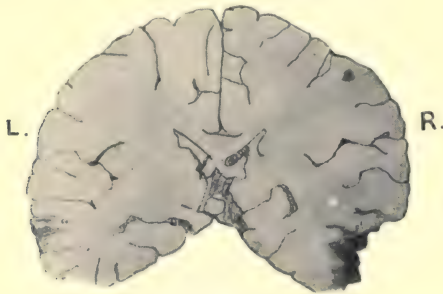


Fig. 25 (Case 30).—Coronal section (posterior surface) through the temporal lobes, showing the depth of the direct and contrecoup bruises.

Postmortem examination revealed a linear fracture of the right posterior fossa, 8 cm. long, contrecoup bruises of the right frontal and temporal lobes and of the left occipital lobe, 40 gm. of blood on the right side behind (extradural) and a bruise of the scalp at theinion.

CASE 9.—A man, aged 42, while intoxicated, was tripped by a boy, October 9, and taken to the Cook County Hospital, where acute alcoholism and alcoholic dementia were diagnosed, and where he remained until October 22, when he was sent to Oak Forest (an infirmary). October 24, he was readmitted to the Cook County Hospital and lived for two days. Skull fracture was diagnosed on the second entrance.

6. Such unfortunate occurrences as these are less likely to be repeated, since they led to the institution of observation rooms at the Cook County Hospital, adjacent to the admittance department, where patients are kept over night or until the presence or nature of injuries is definitely ascertained.

Postmortem examination revealed a comminuted fracture of the right posterior fossa through the right petrous bone, in addition to contrecoup bruises of both temporal lobes, that of the left, 9 by 3 cm., and reaching to the lateral ventricle, into which bleeding had occurred; also there was an extradural clot covering the right parietal lobe weighing 28 gm. There was no external sign of scalp injury. The extradural clots were brown and firmly clotted, the subdural mostly on the left side and weighing only a few grams.



Fig. 26 (Case 31).—Linear fracture of the left middle fossa 9 cm. long. Three days and six hours before death this man, aged 42, fell while in a "fit," and struck his head on a cement sidewalk.

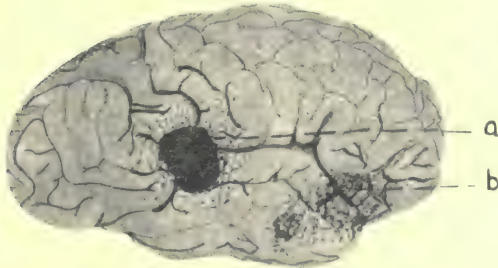


Fig. 27 (Case 31).—A contrecoup bruise at *a* (Class 3) and superficial contusions at *b* (Class 4), with subpial hemorrhage. There were 90 gm. of clotted blood in the subdural space on the right side covering the back half of the hemisphere. There was no bruising of the left cerebral hemisphere.

CASE 10.—A man, aged 35, was struck by another man in a fight. It is not known whether the blow was struck with a fist or weapon. He was examined by a physician immediately, who found "nothing wrong except complaint of headache." Suddenly, after two or three hours, he had a "fit" and was taken to the Hospital of the House of Correction, where he lived two days.

Postmortem examination revealed a depressed, comminuted fracture of the right temporal bone, 120 gm. of blood extradural on the right side in the parietotemporal region, and a superficial bruise of the outside of the right parietal lobe. There were no external injuries, only a swelling in the right temple.

CASE 11.—A boy, aged 8 years, run over by an automobile, November 9, was treated at home until November 28, when he was taken to a private hospital and epidemic meningitis diagnosed. The same day he was removed to the Cook

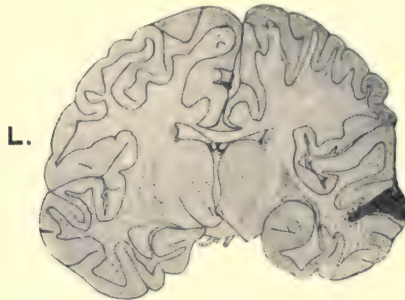


Fig. 28 (Case 31).—Coronal section (posterior surface) through the deepest part of the contrecoup bruise. The symmetry of the brain is due to compression by the subdural clot on the right side.

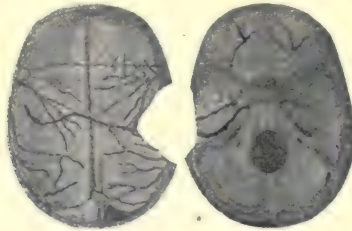


Fig. 29 (Case 32).—Traumatic, comminuted and depressed fracture of the vault and base of the skull with independent fractures of the frontal fossae; also a decompression defect in which five loose pieces of bone were found by the surgeon. The superior longitudinal sinus was ruptured, and extradural bleeding resulted on both sides; that of the left was removed at the operation, and 60 gm. were found on the right side at the postmortem examination. The patient, a white man, aged 25, lived eleven hours and thirty minutes after being hit on the left side of the vault by a heavy iron bar during a fight.

County Hospital by request of the health department, where he lived two days, and epidemic meningitis was again diagnosed. No one suspected skull fracture.

Postmortem examination revealed a small fracture of the ethmoid plate, superficial contusions (mostly subpial hemorrhage) of the frontal poles and left occipital lobe, and men-



ingitis of the base of the brain (pneumococcus). Above the left eyebrow there was a scar.

CASE 12.—A man, aged 37, was in a fight, December 26, and fell to the sidewalk. He was taken to a private hospital, where he remained four days and where laceration of the scalp was diagnosed. The physicians thought there was no skull fracture. He was discharged and went home, but, December 31, he suddenly became very sick and was taken to the Cook County Hospital, where he lived eighteen hours and where skull fracture was diagnosed.

Postmortem examination revealed a fracture of the mid-line of the posterior fossae, and through the right petrous bone; in addition there were also superficial contusions of the tips of the frontal lobes, meningitis of the base, and a healing laceration of the scalp at theinion.

CASE 13.—A man, aged 71, walked into a private hospital

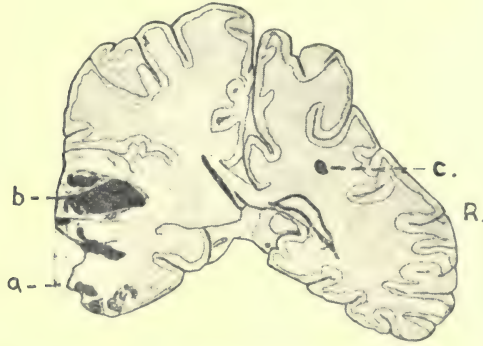


Fig. 30 (Case 32).—Coronal section (posterior surface) through the brain 1 cm. posterior to the optic chiasma showing a tear (Class 2) at *a*, intracerebral bleeding from a bruise anterior to *b*, and the saucer-shape compression of the right cerebral hemisphere by the extradural clot weighing 60 gm. Traumatic hemorrhage of Class 7 at *c*.

at 5:15 p. m. with lacerations of the back of the head, which were dressed there and which he did not know how he received. He walked out of the hospital. Two days later he was arrested and taken to the Hospital of the House of Correction, where he lived four days. Here he was irrational and unable to talk; alcoholism and delirium tremens were diagnosed.

Postmortem examination revealed a fracture of the left middle fossa, linear and 10 cm. long; also direct bruises of the left temporal and parietal lobes covered by adherent, brown subdural clots, weighing about 5 gm.

CASE 14.—A man, aged 48, fell from a moving wagon, January 20, and was taken home. On the 24th he went to a

physicians office, and the physician thought he was suffering from alcoholism. He remained at home for three or four days more, wildly delirious and all this time in bed. He was then taken to the Hospital of the House of Correction, where acute alcoholism was diagnosed and where he lived two days.

Postmortem examination revealed a fracture of the mid-line of the posterior fossae, 39.2 cm. long, deep contrecoup bruises of the frontal and temporal lobes, large subdural clots about the bruises, an extradural clot in the left occipital region, and hemorrhages in the pons. There was extensive hemorrhage into the deep tissues of the scalp behind.

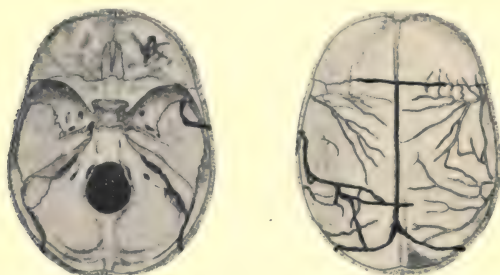


Fig. 31 (Case 33).—Traumatic, extensively comminuted and diastatic fracture of the skull, 123 cm. long, with an independent fracture of the roof of the right orbit. The greatest scalp injury was over the back half of the right parietal bone. This man, aged 35, fell from a ladder head first, about two hours before death.

CASE 15.—A man, aged 45, was found on a sidewalk by the police at 1 a. m., was taken to the police station, where an ambulance surgeon diagnosed alcoholism, and where he remained until 2:40 p. m. the same day, when he was taken to the Hospital of the House of Correction. Here skull fracture was diagnosed, and he lived only thirty-five minutes.

Postmortem examination revealed a fracture of the right middle fossa 18.5 cm. long, 200 gm. of blood extradural in the right temporoparietal region, superficial bruises of the outside of the right temporal lobe, and a thin clot of blood covering the right temporal lobe. There was no external sign of injury.

CASE 16.—A man, aged 49, found on the street by the police at 1 a. m., was taken to the police station, where he remained four hours, and then to a private hospital, where a physician diagnosed skull fracture and alcoholism. He was then transferred to the Hospital of the House of Correction at 6 a. m., and died the same day at 7:40 a. m. Skull fracture was diagnosed in the latter hospital.

Postmortem examination revealed a fracture of the right posterior fossa and traumatic diastasis of the right lamb-

doid suture, altogether 24 cm. long, in addition to contrecoup bruises of both frontal and the left temporal lobes, all superficial except the latter, which reached to the lateral ventricle; there was blood in all of the ventricles of the brain. There was only a little subdural hemorrhage about the bruises. The scalp was lacerated at theinion.

CASE 17.—A man, aged 44, was arrested at 2 a. m. and taken to a police cell, where he remained until 11 a. m. the next day, the ambulance physician having diagnosed incipient delirium tremens. He became violent and was taken to the Hospital of the House of Correction, where he lived eighteen hours, and where delirium tremens was again diagnosed.

Postmortem examination revealed a linear fracture of the left lambdoid suture, right parietal bone and right middle fossa; there were also a contrecoup laceration of the left

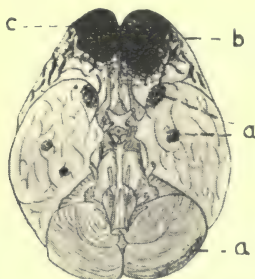


Fig. 32 (Case 33).—Multiple superficial, contrecoup contusions (Class 4) at *a*, a contrecoup tear of the undersurface of the left frontal lobe at *b* (Class 1), and a contrecoup bruise of the right frontal lobe (Class 2) at *c*. There were 190 gm. of blood in the left subdural space covering the entire left cerebral hemisphere, and only a few grams on the right side at *c*.

frontal lobe 4.5 cm. deep, a large subdural clot covering the top and side of the left frontal lobe, a small extradural clot in the right middle fossa, and hemorrhage into the right eyelid. There was no other sign of injury of the scalp, but in the deep scalp tissues of the right parietal region there was hemorrhage.

CASE 18.—A man, aged 24, while trying to escape the police, jumped off a moving train into the Drainage Canal. He was taken to the Hospital of the House of Correction, where no diagnosis was made and where he stayed for one week. At home he complained of headache, and after ten days was removed to the Cook County Hospital, where purulent meningitis was diagnosed and where he lived one day.

Postmortem examination revealed a fracture of the ethmoid plate and sella turcica, superficial contusions of both frontal lobes, and meningitis of the base.

CASE 19.—A man, aged 53, was found intoxicated by the police, 8:15 p. m., June 6; the police took him to a police station, where he fell on the floor; the wounds sustained were dressed at a private hospital, and he was again taken to the police station, where he remained until 11:30 p. m., June 7. He was then removed to the Hospital of the House of Correction, where he died, June 10, at 12 m. Basal skull fracture was diagnosed.

Postmortem examination revealed a fracture of the mid-line of the vault and posterior fossae, 50 cm. long; also there were extensive contusions of the undersurfaces of the

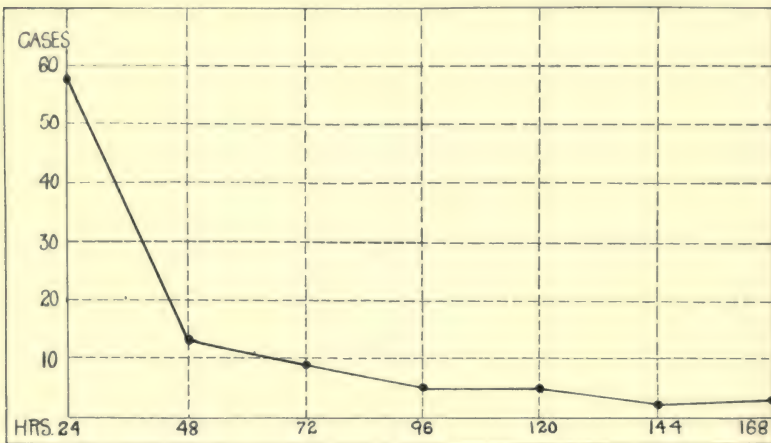


Fig. 33.—Time of death of ninety-five of the 104 patients dying with large extradural hemorrhages. Of the nine not shown in this graph, five were found dead and four died between the eighth and the twenty-second day after entrance to hospital. (Other details connected with some of these extradural hemorrhages, diagnosis, operations, etc., are given in the article by Dr. Moody.)

frontal and temporal lobes of both sides, 16 gm. of blood subdural at the bruises, 30 gm. extradural along the superior longitudinal sinus, and meningitis of the left temporal lobe.

CASE 20.—A man, aged 70, was arrested for acute alcoholism and kept in a police station for ten hours; then he was removed to the Hospital of the House of Correction, where he remained two days; lobar pneumonia and delirium tremens were diagnosed.

Postmortem examination revealed a fracture of the right middle fossa, 17.5 cm. long, a large laceration of the right parietal and temporal lobes reaching to the lateral ventricle, 75 gm. of blood outside the dura and covering the right parietal region, and extensive hemorrhage into the right side of the scalp behind.



Other cases, such as these, concern cranial fracture recognized by physicians, and the patients transferred from one hospital to another:

CASE 21.—A man, aged 62, was run over by an automobile about one hour before entrance to a hospital, where he was given first aid; he was then taken to another hospital, where a decompression operation was performed above the right ear; here he remained for three or four days, and was then taken to the Cook County Hospital, where he lived three and one-half days and where old skull fracture, infected decompression wound and fractured ribs were diagnosed.

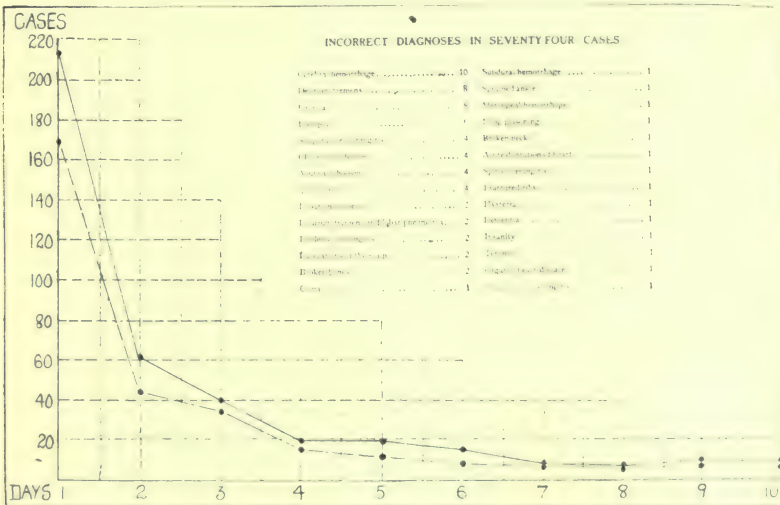


Fig. 34.—Time in twenty-four hour periods of death from fracture of the cranial bones and brain injuries of 403 of the 504 patients (upper line), and number of correct diagnoses during the same periods (lower line). The 101 remaining are not included because the time for eighteen of them was from eleven to twenty-two days, inclusive; twenty-seven had to do with persons found dead; for thirty-eight the records were incomplete; and eighteen concerned healed fractures. Any consideration of diagnosis must take into account the time under observation. Therefore, such charts as were used by Bissell and Lecount (A Consideration of the Relative Frequency of the Various Forms of Coma, *THE JOURNAL*, March 27, 1915, p. 1041; Feb. 17, 1917, p. 500) are continued here.

Postmortem examination revealed a fracture through the right petrous bone, 18 cm. long, superficial contrecoup bruises of the frontal and temporal lobes, fibrinopurulent meningitis of the top of the brain, and purulent ependymitis.

CASE 22.—A man, aged 37, was struck by a street car at midnight, December 18, and taken to a private hospital, where skull fracture was diagnosed, and where he remained

for five days. Because of inability to assure payment for hospital service, the patient was transferred to the Cook County Hospital, where he lived until December 29, altogether eleven days.

Postmortem examination revealed a linear fracture of the midline of the base and left petrous bone, 38.5 cm. long; there were also lacerations of both frontal lobes, contusions of the temporal lobes and a direct bruise of the undersurface of the cerebellum, a few small subdural clots at the lacerations, and fibrinopurulent meningitis of the cerebellum.

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# TRAUMATIC FRACTURE OF THE CRANIAL BONES

CLINICAL CONSIDERATIONS, WITH ESPECIAL  
REFERENCE TO EXTRADURAL  
HEMORRHAGE \*

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Between August, 1911, and June, 1918, 908 patients entered the Cook County Hospital for "skull fracture." This diagnosis was made certain for 547 of these either by operation or by postmortem examination. Fracture of the cranial bones was diagnosed by means of the roentgen ray for 105 others. The remainder (256) include patients whose illness and injuries were recovered from, and the conclusion that they also suffered from traumatic fracture of the cranial or other skull bones was not as adequately confirmed; for some small part of these, especially those in the hospital only a few hours, the diagnosis may have been wrong. Postmortem examination was made in all deaths but sixty-two; in forty-one of these no fracture of the skull was actually demonstrated.

These considerations, as well as others shown in the accompanying table, are simply prefatory, since it is proposed to discuss here the extradural traumatic middle meningeal hemorrhages. Of the total 908 thought to be skull fractures (547 demonstrated as such), there were 100 with extradural hemorrhages of such size that compression of the brain by the blood was the chief cause of death. This condition was established for all the 100 patients either by operations, by postmortem examinations, or by both.

Diagnosis of traumatic extradural middle meningeal hemorrhage, found postmortem, was not made clinically in sixty-three instances. The time under observation of these sixty-three patients, whose compression of the brain was not recognized, is shown in Chart 1. It will be noted that twenty-four were in the hospital two days or more, the longest period for any single patient being twelve days.

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\* From the Cook County Hospital

Among other details regarding these extradural hemorrhages, shown in the table, is that all of the thirty-seven patients in whom the condition was recognized had a decompression operation. Of this number, twenty-six died, twenty-one being operated on within

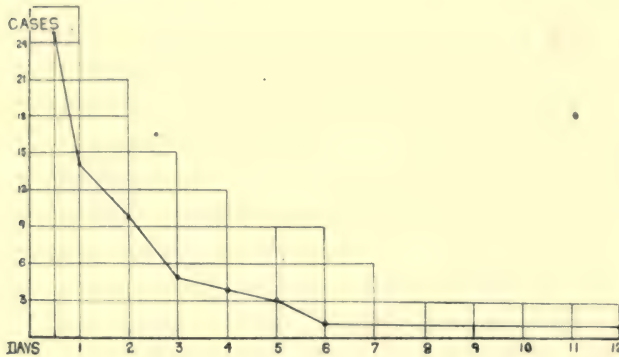


Chart 1.—Number of days under observation of sixty-three patients with extradural hemorrhage found at postmortem examination but not recognized clinically.

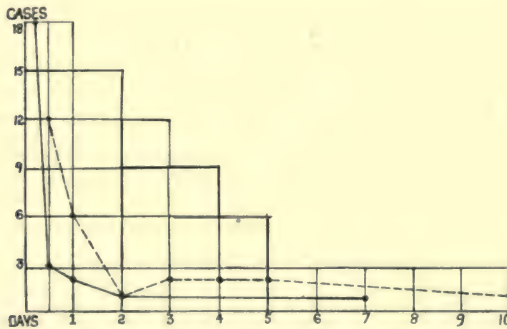


Chart 2.—Time under observation before operation (solid line), and time between operation and death (broken line), of twenty-six patients with extradural hemorrhages, diagnosed and operated on. One patient dying on the twenty-second day with lobar pneumonia is not represented.

twelve hours after entrance to the hospital (Chart 2). The longest period any one patient was under observation before operation was seven days. Following operation, eighteen died within twenty-four hours, three inside of the next two days, and one patient on the twenty-second day (lobar pneumonia).



As stated, sixty-three of the extradural hemorrhages were not recognized clinically. The maximum weight of the extradural blood found postmortem was 246 gm., the minimum, 40 gm., with an average of 110.5 gm. for the sixty-three. In the clinical records for all but two there were symptoms generally regarded as useful in the recognition of compression of the brain; nine patients had hemiplegia, nine rigidity of the muscles on one side, and nine had convulsions; in twenty-three there was a positive Babinski reflex, and in some of the sixty-three the symptoms were combined in various ways.

FINDINGS IN FIVE HUNDRED AND FORTY-SEVEN CASES OF  
SKULL FRACTURE \*

	Fracture Demon- strated	Fracture Not Demon- strated	Extra- dural Hemor- rhage
Died (male).....	407	34	77
Died (female).....	37	7	12
Recovered (male).....	94	285	10
Recovered (female).....	9	35	1
Necropsies.....	423	...	85
Operated on and died.....	82	...	26
Operated on and recovered.....	55	...	11
Entered conscious.....	108	164	13
Entered unconscious.....	436	194	87
Convulsions.....	66	19	14
No clinical evidence of injury.....	80	55	17
Positive Babinski reflex.....	156	64	33
Patellar reflex absent.....	119	42	26
Patellar reflex exaggerated.....	103	59	26
Hemiplegia.....	49	7	21
Spasticity.....	31	7	15
Facial paralysis.....	36	19	8
Ptosis of eyelids.....	12	3	2
Clear cerebrospinal fluid (taken before death).....	25	20	7
Meningitis.....	39	2	0
Bleeding from nose.....	163	107	24
Bleeding from mouth.....	59	43	6
Bleeding from ears.....	132	118	23
Eye reflexes normal.....	156	203	29

\* In sixty-two deaths, no postmortem examination was made. In twenty-one of these, fracture had been previously demonstrated. Roentgenologic diagnosis of skull fracture was made in 133 instances, 105 not being subsequently confirmed (by operation or postmortem examination). There was no roentgenographic evidence of fracture of the skull in ninety-six instances. Seventeen of these were found at necropsy to be fracture.

In general, the pulse at the time of entrance and for a short time afterward was full and strong, from 65 to 90, the temperature from 97 to 99 F. The most conspicuous features of pulse and temperature records, however, are that in the eleven recovering with decompression operation, the pulse and temperature were both lower than in other patients, and this pertains equally to conditions before and after operation.

In twenty-seven of the clinical records blood pressure is recorded, without, however, anything of importance to comment on here because the observations were not repeatedly made for any single patient.

The other important features of these head injuries with one exception are indicated in the table. This exception concerns the records of blood examinations, and of all these 107 were found in the entire number (908). That there was an average leukocytosis of 15,300 apparently makes this condition of importance in the recognition of skull fracture. The number is so large that it is difficult to be accounted for by bronchopneumonia, and still less readily by meningitis; moreover, many of these blood examinations were made shortly after entrance.

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